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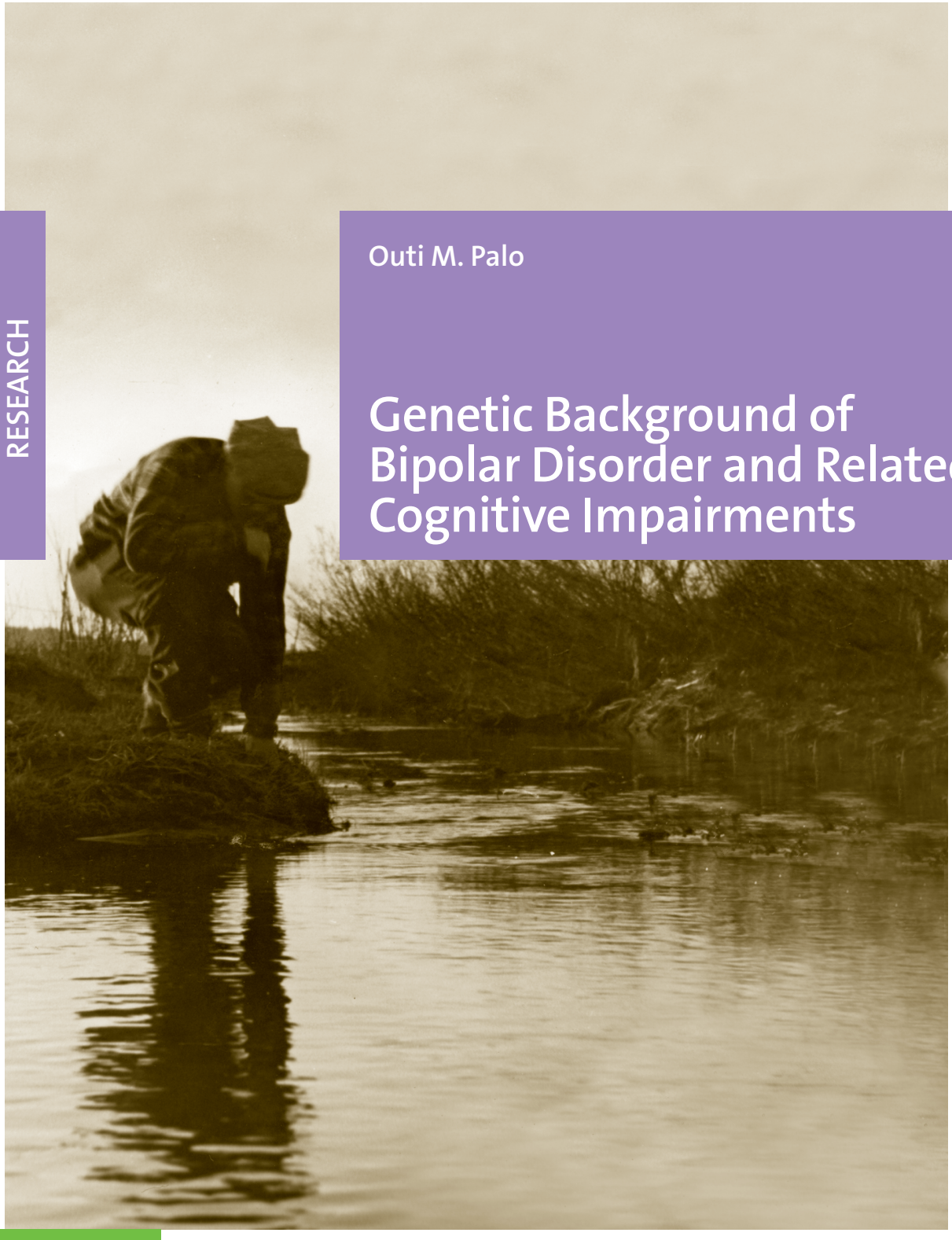

RESEARCH

Outi M. Palo

Genetic Background of Bipolar Disorder and Related Cognitive Impairments

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Genetic Background of Bipolar Disorder and Related Cognitive Impairments

ACADEMIC DISSERTATION

To be presented with the permission of the Medical Faculty, University of Helsinki, for public examination in the Lecture Hall 2, Biomedicum Helsinki, on April 9th 2010, at 12 noon.

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To my family

Abstract

Outi M. Palo. Genetic Background of Bipolar Disorder and Related Cognitive Impairments. National Institute for Health and Welfare (THL), Research 29, 140 pages. Helsinki 2010. ISBN 978-952-245-243-6 (print); 978-952-245-244-3 (pdf)

Bipolar disorder (BP) is a complex psychiatric disorder characterized by episodes of mania and depression. BP affects approximately 1% of the world's population and shows no difference in lifetime prevalence between males and females. BP arises from complex interactions among genetic, developmental and environmental factors, and it is likely that several predisposing genes are involved in BP. The genetic background of BP is still poorly understood, although intensive and long-lasting research has identified several chromosomal regions and genes involved in susceptibility to BP.

This thesis work aims to identify the genetic variants that influence bipolar disorder in the Finnish population by candidate gene and genome-wide linkage analyses in families with many BP cases. In addition to diagnosis-based phenotypes, neuropsychological traits that can be seen as potential endophenotypes or intermediate traits for BP were analyzed.

In the first part of the thesis, we examined the role of the allelic variants of the *TSNAX/DISC1* gene cluster to psychotic and bipolar spectrum disorders and found association of distinct allelic haplotypes with these two groups of disorders. The haplotype at the 5' end of the Disrupted-in-Schizophrenia-1 gene *(DISC1)* was over-transmitted to males with psychotic disorder ($p = 0.008$; for an extended haplotype $p = 0.0007$ with both genders), whereas haplotypes at the 3' end of *DISC1* associated with bipolar spectrum disorder ($p = 0.0002$; for an extended haplotype $p = 0.0001$). The variants of these haplotypes also showed association with different cognitive traits. The haplotypes at the 5' end associated with perseverations and auditory attention, while the variants at the 3' end associated with several cognitive traits including verbal fluency and psychomotor processing speed.

Second, in our complete set of BP families with 723 individuals we studied six functional candidate genes from three distinct signalling systems: serotonin-related genes (*SLC6A4* and *TPH2*), BDNF -related genes (*BDNF*, *CREB1* and *NTRK2*) and one gene related to the inflammation and cytokine system (*P2RX7*). We replicated association of the functional variant Val66Met of *BDNF* with BP and better performance in retention. The variants at the 5' end of *SLC6A4* also showed some evidence of association among males ($p = 0.004$), but the widely studied functional variants did not yield any significant results. A protective four-variant haplotype on *P2RX7* showed evidence of association with BP and executive functions: semantic and phonemic fluency ($p = 0.006$ and $p = 0.0003$, respectively).

Third, we analyzed 23 bipolar families originating from the North-Eastern region of Finland. A genome-wide scan was performed using the 6K single nucleotide polymorphism (SNP) array. We identified susceptibility loci at chromosomes 7q31 with a LOD score of 3.20 and at 9p13.1 with a LOD score of 4.02. We followed up both linkage findings in the complete set of 179 Finnish bipolar families. The finding on chromosome 9p13 was supported (maximum LOD score of 3.02), but the susceptibility gene itself remains unclarified.

In the fourth part of the thesis, we wanted to test the role of the allelic variants that have associated with bipolar disorder in recent genome-wide association studies (GWAS). We could confirm findings for the *DFNB31, SORCS2, SCL39A3*, and *DGKH* genes. The best signal in this study comes from *DFNB31*, which remained significant after multiple testing corrections. Two variants of *SORCS2* were allelic replications and presented the same signal as the haplotype analysis. However, no association was detected with the *PALB2* gene, which was the most significantly associated region in the previous GWAS.

Our results indicate that BP is heterogeneous and its genetic background may accordingly vary in different populations. In order to fully understand the allelic heterogeneity that underlies common diseases such as BP, complete genome sequencing for many individuals with and without the disease is required. Identification of the specific risk variants will help us better understand the pathophysiology underlying BP and will lead to the development of treatments with specific biochemical targets. In addition, it will further facilitate the identification of environmental factors that alter risk, which will potentially provide improved occupational, social and psychological advice for individuals with high risk of BP.

Keywords: Bipolar disorder, complex disease, linkage analysis, association analysis, cognition

Abstract in Finnish

Outi M. Palo. Genetic Background of Bipolar Disorder and Related Cognitive Impairments [Kaksisuuntaisen mielialahäiriön geneettinen tausta ja siihen liittyvät kognitiiviset häiriöt]. Terveyden ja hyvinvoinninlaitos (THL), Tutkimus 29, 140 sivua. Helsinki 2010.
ISBN 978-952-245-243-6 (painettu), ISBN 978-952-245-244-3 (pdf)

Kaksisuuntainen mielialahäiriö (BP) on monitekijäinen psykiatrinen sairaus, jolle on tyypillistä maanisten ja depressiivisten jaksojen toistuva vaihtelu. Kaksisuuntaiseen mielialahäiriöön sairastuu noin 1 % koko maailman väestöstä ja sairautta esiintyy yhtälailla miehillä kuin naisilla. Kaksisuuntaisen mielialahäiriön puhkeamiseen vaikuttavat geneettiset tekijät yhdessä kehitys- ja ympäristötekijöiden kanssa ja useat geenit todennäköisesti altistavat sairastumisriskiin. Kaksisuuntaiseen mielialahäiriön geenitaustan tunnemme kuitenkin vielä huonosti, vaikka intensiivisen ja pitkäaikaisen tutkimuksen avulla olemme tunnistaneet useita kromosomaalisia alueita ja geenejä, joiden arvellaan vaikuttavan kaksisuuntaiseen mielialahäiriön alttiuteen.

Tässä väitöskirjatutkimuksessa on pyritty paikantamaan geenivariantteja, jotka altistavat kaksisuuntaiseen mielialahäiriöön suomalaisessa väestössä kandigeenianalyysin ja genomin laajuisen kytkentäanalyysin avulla. Diagnostisen luokituksen lisäksi analysoimme suoriutumista neuropsykologisissa testeissä ja näiden piirteiden mahdollista assosioitumista geenivariantteihin. Neuropsykologiset testit mittaavat kognitiivisia ominaisuuksia, jotka mahdollisesti liittyvät sairauteen, mutta ovat sen puhkeamisesta riippumattomia piirteitä eli endofenotyyppejä.

Väitöskirjan ensimmäisessä osassa tavoitteena oli tutkia *TSNAX/DISC1* geenialueen varianttien merkitystä psykoottisen ja bipolaarispektrin sairauksille. Erilaisilla alleelisilla haplotyypeillä havaitsimme olevan vaikutus näihin molempiin sairauksiin. *DISC1* geenin 5'-päässä olevan haplotyypin todettiin periytyneen psykoosiin sairastuneille miehille ($p = 0.008$; laajennetulle haplotyypille, $p = 0.0007$ molemmille sukupuolille), kun taas *DISC1* geenin 3'-päässä olevan haplotyypin todettiin assosioituvan bipolaarispektrin sairauteen ($p = 0.0002$ laajennetulle haplotyypille, $p = 0.0001$). Lisäksi haplotyypin variantit osoittautuivat assosioituvan useisiin kognitiivisiin piirteisiin. *DISC1* geenin 5'-päässä olevan haplotyypin todettiin assosioituvan useisiin kognitiivisiin piirteisiin, kuten toistovirheisiin ja kuulonvaraiseen tarkkaavaisuuteen. Kun taas 3' päässä olevat variantit assosioituivat kielelliseen sujuvuuteen ja psykomotoriseen prosessointinopeuteen.

Geenit, jotka liittyvät kolmeen täysin erilaiseen signallointisysteemiin, joiden tehtävänä on säädellä monia keskushermoston tehtäviä, on osoitettu altistavan kaksisuuntaiselle mielialahäiriölle. Tässä tutkimuksessa tutkimme kuutta funktionaalista kaksisuuntaiseen mielialahäiriön kandigeeniä; serotoniiniin liittyviä geenejä

(*SLC6A4* and *TPH2*), *BDNF:n* liittyviä geenejä sekä yhtä tulehdus ja sytokiiniinisysteemiin liittyvää geeniä (*P2RX7*) 723 suomalaisella bipolaariperheen-jäsenellä. *BDNF:n* funktionaalinen variantti Val66Met assosioitui kaksisuuntaiseen mielialahäiriöön ja parempaan suoriutumiseen muistiaineksen mielessä säilymisessä. Myös *SLC6A4* geenin 5'–päässä olevien varianttien assosiaation pystyimme toistamaan erityisesti miehillä ($p = 0.004$), mutta assosiaatiota laajasti tutkituille funktionaalisille varianteille emme havainneet. Suojaava neljän variantin haplotyyppi geenissä *P2RX7* assosioitui kaksisuuntaiseen mielialahäiriöön sekä toiminnan ohjaukseen, erityisesti foneemisessa ($p = 0.0003$) ja semanttisessa ($p = 0.006$) sanasujuvuudessa.

Väitöskirjan kolmannessa osatyössä tutkittiin 23 bipolaariperhettä, jotka ovat kotoisin Suomen koillisosasta. 6K varianttia testattiin genominlaajuisella kytkentäanalyysillä. Merkittävimmät kytkentälöydökset havaitsimme kromosomialueella 7q31 (LOD = 3.20) ja 9p13.1 (LOD = 4.02) havaittiin kromosomialueella 7q31. Jatkotutkimuksena analysoimme molemmat kytkentälöydökset parhaimmilla markkereilla 179 perheellä. Kromosomialueen 9p13 kytkentälöydöksen pystyimme toistamaan tässä aineistossa LOD-arvolla 3.02.

Väitöskirjan viimeisessä osatyössä testattiin genominlaajuisissa assosiaatiotutkimuksissa löydettyjen alleelisten varianttien assosioitumista kaksisuuntaiseen mielialahäiriöön. Pystyimme toistamaan löydökset *DFNB31, SORCS2, SCL39A3*, ja *DGKH* geeneille. Merkittävin tulos tuli geenistä *DFNB31*, mikä säilyi merkittävänä myös moninkertaisen testauksen korjaamisen jälkeen. *SORCS2*-geenin varianteista kaksi olivat alleelisia replikaatioita, jota tuki myös haplotyyppianalyysi. Aiemmissa koko genominlaajuisissa assosiaatioanalyyseissä merkittävimmän tuloksen antaneelle *PALB2* geenille emme löytäneet näyttöä sen osuudesta kaksisuuntaiseen mielialahäiriöön suomalaisessa väestössä.

Tämän väitöskirjatyön tulokset ovat osoittaneet kaksisuuntaiseen mielialahäiriön olevan heterogeeninen ja sen geneettisen taustan vaihtelevan eri väestöjen välillä. Jotta voimme täysin ymmärtää kompleksitautien, kuten kaksisuuntaiseen mielialahäiriöön, taustalla olevien geneettisten tekijöiden alleelista heterogeenisyyttä edellytetään usean terveen ja sairastuneen henkilön koko genomin sekvensointia. Tiettyjen alttiusgeenien löytäminen auttaisi ymmärtämään kaksisuuntaiseen mielialahäiriön tautimekanismia ja näin ollen mahdollistaisi paremmin tehoavien lääkkeiden kehittämistä. Lisäksi tällöin olisi myös mahdollista tunnistaa ympäristötekijöitä, jotka vaikuttavat alttiuteen sairastua. Perintö- ja ympäristötekijöiden tunnistaminen auttaisi myös järjestämään asianmukaista ammatillista, sosiaalista ja psykologista tukea henkilöille, joilla on korkea riski sairastua kaksisuuntaiseen mielialahäiriöön.

Avainsanat: kaksisuuntainen mielialahäiriö, monitekijäinen tauti, kytkentäanalyysi, assosiaatioanalyysi, kognitio

Contents

List of original papers

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals:

I ***Outi M. Palo***, Mervi Antila, Kaisa Silander, William Hennah, Helena Kilpinen, Pia Soronen, Annamari Tuulio-Henriksson, Tuula Kieseppä, Timo Partonen, Jouko Lönnqvist, Leena Peltonen, Tiina Paunio. Association of distinct allelic haplotypes of DISC1 with psychotic and bipolar spectrum disorders and with underlying cognitive impairments. Hum Mol Genet. 2007 Oct 15;16(20):3517–28.

II Pia Soronen, ***Outi M. Palo***, Mervi Antila, Annamari Tuulio-Henriksson, Kaisa Silander, Tuula Kieseppä, Anu Loukola, Jouko Lönnqvist, Leena Peltonen, Timo Partonen, Tiina Paunio. Evidence for BDNF, SLC6A4 and P2RX7 association with bipolar disorder. (Submitted)

III ***Outi M. Palo***, Pia Soronen, Kaisa Silander, Teppo Varilo, Katja Tuononen, Tuula Kieseppä, Timo Partonen, Jouko Lönnqvist, Tiina Paunio, Leena Peltonen. Identification of susceptibility loci at 7q31 and 9p13 for bipolar disorder in an isolated population. Am J Med Genet B Neuropsychiatr Genet. 2009. In press.

IV Hanna M. Ollila, Pia Soronen, Kaisa Silander, ***Outi M. Palo***, Tuula Kieseppä, Mari A. Kaunisto, Jouko Lönnqvist, Leena Peltonen, Timo Partonen, Tiina Paunio. Findings from bipolar disorder genome-wide association studies replicate in a Finnish bipolar family-cohort. Mol Psychiatry. 2009 Apr;14(4):351–3.

Abbreviations

ADHD	Attention –deficit/hyperactivity disorder
ALDH1B1	Aldehyde dehydrogenase 1B1 precursor
ANK3	Ankyrin G
ARNTL	Aryl hydrocargon receptor nuclear translocator-like gene
BP	Base pair
BP	Bipolar disorder
BP-I	Bipolar disorder, type 1
BP-II	Bipolar disorder, type 2
BP-NOS	Bipolar disorder, not otherwise specified
BDNF	Brain-derived neurotrophic factor
CACNA1C	Alpha 1C subunit of the L-type voltage-gated calcium channel
CADPS2	Calcium-dependent activator protein for secretion 2
Caspr3	Contactin-associated protein 3
CLOCK	Circadian locomotor output cycles kaput
cM	Centimorgan
CNTNAP3	Contacting associating protein-like 3
CNV	Copy number variants
COMT	Catechol-O-methyltransferase
COWAT	The Controlled Oral Word Association Test
CREB1	cAMP responsive element binding protein 1
CD-CV	Common disease-common variant
CVLT	Californian Verbal Learning Test
DAOA	D-amino acid oxidase activator or G72 /G30
DFNB31	CASK-interacting protein CIP98 isoform 1
DGKH	Diacylglycerol kinase, eta
DISC1	The Disrupted-in-Schizophrenia-1 gene
DNA	Deoxyribonucleic acid
DSM-III-R	The Diagnostic and Statistical Manual of Mental Disorders, revised third edition
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, fourth edition
DPY19L3	Dpy-19-like 3
DZ	Dizygotic
DISC1	Disrupted-in-Schizophrenia 1
EDTA	Ethylenediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
FBAT	Family–based association test
GSK3-β	glycogen synthase kinase 3-β gene
GWAS	Genome-wide association studies
HBAT	The haplotype extension of the FBAT program
hME	Homogenous Mass Extension
HGP	Human Genome Project
HRR	Haplotype relative risk
ICD-8	The International Classification of Diseases, version 8

ICD-10	The International Classification of Diseases, tenth revision
KCND2	Potassium-voltage-gated channel, member 2
Kb	Kilobase
LD	Linkage disequilibrium
LOD	Logarithm of odds
MAOA	Monoamine oxidase A
Mb	Megabase
mRNA	Messenger RNA
MYO5B	Myosin 5B
MZ	Monozygotic
NAP5	Nck-associated protein 5
NPL	Non-parametric linkage
NT-3	Neurotrophin-3
NTRK2	Neurotrophic tyrosine kinase, receptor type 2
OR	Odds ratio
PALB2	Partner and localizer of BRCA2
PCR	Polymerase chain reaction
PER3	Period homolog 3
PLA2A	Phospholipase A2
PLAP	Phospholipase A2-activating protein
P2RX7	Purinergic receptor ligand-gated ion-channel 7
QTDT	Quantitive Transmission Disequilibrium test
rMDD	Recurrent major depression disorder
RNA	Ribonucleic acid
SA	Schizoaffective disorder
SAD	Seasonal affective disorder
SCID	Structured clinical interview
SCZ	Schizophrenia
SLC6A4	Solute carrier family 6 member 4
SLC39A3	Solute carrier family 39
SNP	Single nucleotide polymorphism
SORCS2	Sortilin-related VPS10 domain containing receptor 2
STR	Short tandem repeat
TDT	Transmission disequilibrium test
θ	Theta, recombination fraction
TPH2	Tryptophan hydroxylase 2
TSNAX	Translin-Associated factor X
TSPAN8	Tetraspanin-8
UPD	Unipolar disorder
WAIS-R	The Wechsler Adult Intelligence Scale-Revised
WHO	World Health Organization
WMS-R	The Wechsler Memory Scale-Revised
WTCCC	The Wellcome Trust Case Control Consortium
Z	Lod score

Gene names have been italicized in the text.

1 Introduction

Bipolar disorder (BP) is a severe mental disorder affecting approximately 1% of the world population. The disease is characterized by episodes of mania and depression. The episodes of depression are usually more frequent than the manic episodes, although the nature of the illness varies (Goodwin and Jamison 2007). The aetiology of BP is complex, but family, twin and adoption studies have demonstrated considerably high heritability of the disorder (Berrettini 2000a; Berrettini 2000b). The highest estimate, 93 %, is based on the analysis of a Finnish twin study (Kieseppa et al. 2004), suggesting genetic factors play a strong role in BP pathogenesis in Finland. However, the importance of environmental effects are inferred from the relatively high discordance rate in monozygotic twins (Goodwin and Jamison 2007).

BP is recognized as a complex disease that is thought to be to the result of the collective involvement of several genes and the environment. The number and small effects of the predisposing genes makes identifying them challenging.

Strategies for elucidating the specific genetic basis for BP include linkage and associating methods. Two alternative approaches have been commonly employed to identify in BP and other complex disorders. The functional candidate approach is based on the selection of genes that are thought to be involved in the biological processes of the disease. The positional candidate approach, on the other hand, is based on the selection of genes that are located in regions linked or associated to BP in linkage studies and genome-wide association studies.

Using genetically isolated populations in studies of complex diseases can provide a great advantage in the identification of genetic factors contributing to risk of the disorder. The sequence of the human genome is largely known, enabling the identification and characterization of all associated genes. Increased information on molecular markers, improved genotyping technologies and efficient use of bioinformatic tools to analyze the genomic information enable us to better understand the genetic background of complex diseases.

This study aims to investigate the genetic basis of bipolar disorder and to identify susceptibility genes for BP in Finnish families.

2 Review of literature

2.1 Genetic epidemiology of complex diseases

2.1.1 Genome and its variation

James Watson and Francis Crick discovered the structure of deoxyribonucleic acid (DNA) 56 years ago (Watson and Crick 1953) and the variation in the DNA sequences of genomes was recognized at the end of the 1970's (Botstein et al. 1980). The sequencing of the complete human genome and the localization of approximately 20 000 protein coding genes by The Human Genome Project (HGP) was achieved at the beginning of this century (Clamp et al. 2007; International Human Genome Sequencing Consortium 2000; Venter et al. 2001).

The human cell contains mitochondrial and nuclear genomes. The nuclear genome consists of roughly 3.2 billion base pairs (bp) of double stranded DNA that is organized and packed into 22 autosomal chromosomes and sex-determining X and Y-chromosomes. Each individual receives one chromosome of each type from each parent, with the end result of having 22 autosomal chromosomes and a sex chromosome pair of either XX in females or XY in males. The genomic sequences of the protein-coding genes contain protein-coding exons, introns, promoters and other regulatory regions. In addition, the genomic sequence contains non-coding information that is not translated into a protein, but is used as functional elements, such as ribonucleic acid (RNA) molecules, and so-called "junk DNA" whose function is still poorly known (Gerstein et al. 2007) (Figure 1).

Among humans the genome is approximately 99.5 % identical. Any given individual differs genetically 0.1-0.5 % from other humans, which is caused by several types of sequence variation and polymorphisms (Levy et al. 2007). The genetic variation in humans arises from: (1) mutation, including point mutations caused by substitution, deletion or insertion; (2) chromosomal rearrangements, including deletion, duplication, inversion, insertion and translocation; and (3) random assortment of the chromosomes into gametes (Figure 2). Mutations can be silent, alter gene expression or change protein production leading occasionally to changes in phenotypes. If mutations occur in the germ line, they are transmitted to offspring and create variation in the population.

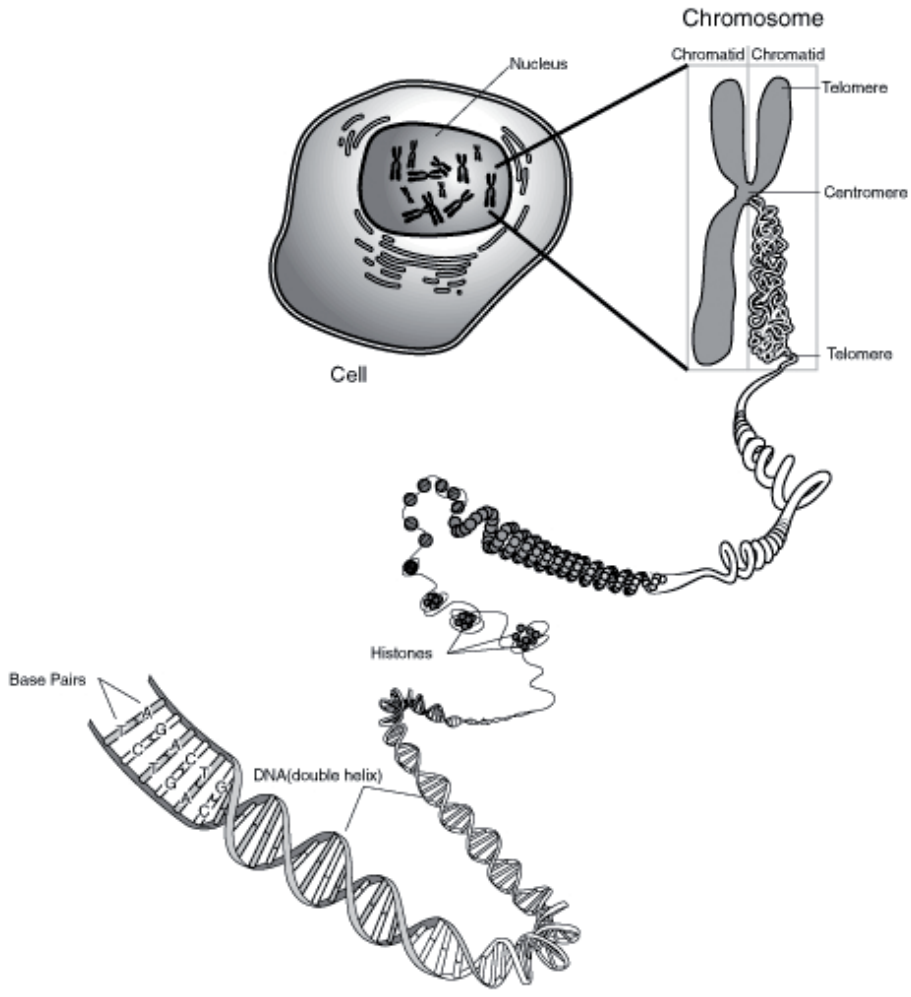

FIGURE 1. The structure of the human genome. The genome is over three billion base pairs of double stranded DNA that is wrapped around histone proteins and tightly packed into 23 chromosome pairs in the nucleus of the cell. The human genome contains an estimated 20,000 protein-coding genes as well as non-coding genes, regulatory sequences, introns and "junk" DNA (http://www.genome.gov/glossary.cfm).

FIGURE 2. The main types of the mutation in the genome.

Short tandem repeats (STRs) or microsatellites have traditionally been the most utilized markers for genome-wide scans (Figure 3). Microsatellites are short di-, tri-, or tetranucleotide tandem repeat sequencesthat are highly polymorphic among individuals (Weber and May 1989). There are over 300 000 known microsatellites in the human genome (NCBI Build 36.2).

Single nucleotide polymorphisms (SNPs) are bi-allelic markers, which are densely spread throughout the human genome, occuring approximately once in every 300 BP (Kruglyak and Nickerson 2001). At present, almost seven million SNPs have been identified and validated (NCBI Build 36.2). Since our knowledge of the human genome sequence is based on only a few sequenced individuals, the number of SNPs is likely underestimated and current estimates exceed 14 million (Kruglyak and Nickerson 2001; Levy et al. 2007). SNPs are conventionally utilized for fine mapping in the positional candidate gene and functional candidate gene approaches. Sets of hundreds of thousands of SNPs on a single array chip have been recently used for screening the whole human genome (Carlson et al. 2004; Hirschhorn and Daly 2005).

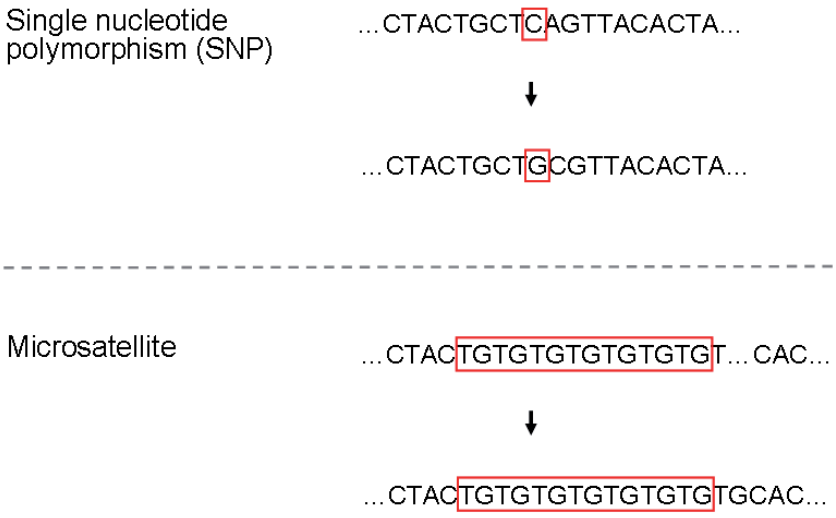

FIGURE 3. The main types of genetic variants utilized in genetic mapping.

Another type of human genome variation is copy number variants (CNVs), which are large genomic regions ranging from a few kilobases (kb) to several megabases (Mb) long that vary in copy number between individuals. CNVs are common and they have been estimated to cover 12 % of the human genome (Redon et al. 2006). CNVs have been shown to influence gene expression and gene dosage being the important structural basis for genetic recombination of the genome.

2.1.2 Strategies to identify genes underlying complex traits

The role of genetic factors in the etiology of diseases varies greatly; some diseases are determined mainly by genes, while others are induced by environment or by gene-environment interactions. Genetic disorders that are caused by highly penetrant mutations in a single gene are called monogenic disorders or Mendelian disorders. Monogenic disorders display clearly identifiable patterns of inheritance (dominant, recessive or X-linked inheritance) and they are usually rare, such as those in the Finnish disease heritage (Norio 2003). Most of the genes responsible for monogenic disorders have been successfully identified by linkage analysis and positional cloning. However, for many complex disorders the identification of predisposing genes has been more challenging (Table 1). Complex disorders are caused by numerous genes interacting with each other and environmental risk factors. Complex disorders, such as bipolar disorder, depression, diabetes, cardiovascular diseases and asthma, affect more people globally than monogenic

disorders. Complex diseases display a complex pattern of inheritance with late onset and incomplete penetrance, and thus they are more likely to be caused by several genes.

Hypotheses of the manner by which allelic variants cause complex diseases differ. The most well-known hypothesis, the common disease-common variant (CD-CV) hypothesis, suggests that common diseases may be caused by a large number of genetic variants with a relatively high frequency in the population (Collins et al. 1997; Lander et al. 2001). The alternative hypothesis, the heterogeneity model, argues that common diseases are caused by several rare variants with strong phenotypic effects (Wang et al. 2005). According to the neutral selection hypothesis, common variants would have low contribution to the genetic variation since they would be close to the fixation in the constant size population (Pritchard 2001; Wang and et al.2005). Currently we do not have enough evidence to determine which of these hypotheses best describes the nature of allelic variants underlying complex diseases. The reality probably lies between these hypotheses with both rare and common variants contributing to susceptibility to complex disorders.

TABLE 1. Complicating factors in the identification of genes predisposing to complex disorders.

Genetic factors	Unknown mode of inheritance Unknown allele frequency Epistasis Genetic heterogeneity Gene-environment interaction
Phenotypes	Difficulties in diagnosis Late onset of the disease Pleiotrophy Phenocopies Incomplete penetrance
Technical issues	Affordable large-scale genotyping methods Completeness of genetic data in public databases
Statistical analyses	Multiple testing Limited statistical power
Publication bias	A tendency to publish significant results, but not negative or neutral results

One strategy to identify susceptibility genes underlying complex disorders is linkage analysis, which aims to identify chromosomal regions linked to disease by following the co-segregation of markers and traits in families. In other words, linkage analysis assesses the linkage probability of two loci on a chromosome by using the frequency of meiotic recombination as an estimate of genetic distance. The recombination fraction, theta (θ), ranges from $\theta = 0$ for loci right next to each

other to $\theta = 0.5$ for loci far apart. A theta $\theta < 0.5$ is observed when two loci co-segregate, and are thus genetically linked. The aim of linkage analysis is to test whether an observed deviation from 50 % recombination between two loci is statistically significant. The statistical significance is measured using a logarithm of odds (LOD) score. LOD scores greater than or equal to 3 are considered to show statistically significant linkage (Ott 1999; Ott and Bhat 1999; Terwilliger and Ott 1993). After genome-wide linkage scans, the identified chromosomal loci are fine mapped by a denser set of markers in order to narrow down the region and identify causative variants.

Association analysis aims to identify alleles that differ systematically between affected and non-affected individuals. This strategy is likely to be more powerful than linkage studies in identifying susceptibility genes with small effects. Association studies can be used in different types of study samples, including case-control samples. Case-control studies are prone to false positive associations caused by population stratification and systematic errors in control sampling. Population stratification refers to the genetically different subgroups in samples that can cause cases and controls to be match poorly genetically. Relatively large sample sizes are required to detect susceptibility variants with only small effects.

In family-based association studies, the Haplotype-relative risk (HRR) method is one method used to compare the frequencies of genotypes and alleles between proband and control groups, the latter being composed of alleles not transmitted to affected individuals. Parental alleles are sorted into transmitted alleles to affected offspring and non-transmitted alleles (Falk and Rubinstein 1987; Ott 1999). A modification of HRR is the transmission disequilibrium test (TDT), which uses data from parents and their affected offspring to test linkage in the presence of linkage disequilibrium (LD). The frequency of transmitted alleles and non-transmitted alleles of parental controls are compared (Spielman and Ewens 1996; Spielman et al. 1993; Spielman et al. 1994). The main disadvantage of the family-based association studies is that parental data is not always available.

The LD method measures if the co-occurrence of two variants at different loci differs from chance occurrence (Kruglyak 1999). The most commonly used measures for LD are D' and r^2, which are scaled such that 0 signifies no LD and 1 to total LD (ie. no recombination). Both measures are independent of allele frequencies and can be used to compare differences in LD between loci (Ardlie et al. 2002; Weiss and Clark 2002). LD results from the inheritence of a particular marker and disease susceptibility allele together as a unit, or haplotype, which over time and after several generations can still be detected (Terwilliger and Goring 2000). As a result, the susceptibility allele will be detected in affected individuals in numerous apparently unrelated individuals and families. Therefore, even when the association of the susceptibility allele is not tested, the other alleles that are in LD with susceptibility allele will be inherited together in the same haplotype more often than expected by chance. Recombinations in the genome do not occur evenly.

Instead, they occur more frequently in recombination hotspots, resulting in block-like structures across the genome. These haploblocks consist of regions with low recombination rates, and thus variants within a block are in considerable LD. For this reason, a few SNPs can be selected for genotyping to obtain information on the adjacent SNPs within these haploblocks. Only these "tagging" SNPs need to be genotyped in order to describe the genetic variation of haploblocks (Hirschhorn and Daly 2005). Although a majority of haplotypes is shared between populations, there are still some parts of the genome that cannot be tagged and individual SNPs need to be genotyped Frazer et al. 2007). The size of LD varies across populations. Due to the population history of Finland, the genome of Finns appears to have increase in LD compared to other populations, which facilitates the gene mapping of complex diseases in the Finnish population (Peltonen et al. 1999; Varilo and Peltonen 2004). The most significant value of isolated populations is the lower genetic variability that results in the enrichment of a subset of predisposing alleles. Enrichment of predisposing alleles will require fewer individuals for the mapping of genes involved in the etiology of the complex disorders. In addition, isolated populations, such as Finns and Sardinians, have similar LD levels to other populations in Europe and the United States (Kaessmann et al. 2002).

The HapMap project was undertaken to catalogue all the common variants with minor allele frequencies of at least 1–5 % in a SNP and haplotype map of the human genome. It was meant to accelerate the discovery of the susceptibility genes underlying common andcomplex diseases (The International HapMap Consortium 2003). Most of the panels used in genome-wide association studies (GWAS) are based on the HapMap data, and therefore it is worth noting that they are designed to detect common variants and may not detect rare genetic variants(Hirschhorn and Daly 2005). The recent GWAS use dense mapping methods covering the genome with 300K-1M SNPs and have large case-control study samples of several thousands of individuals (McCarthy et al. 2008). GWAS have identified common variants showing association with common complex diseases, such as type 2 diabetes (Saxena et al. 2007), Crohn's disease (Rioux et al. 2007) and prostate cancer (Gudmundsson et al. 2007). Replication has become the gold standard for assessing results from GWAS. However, the replication requirement may cause real genetic effects to be missed. A real result can fail to replicate for numerous reasons, such as inadequate sample size or variability in phenotype definitions across independent samples. Failure to replicate may provide important clues about the complexity of the underlying genetic architecture of the disease and non-replicated variants could be checked for interactions with other variants, particularly when samples are collected from different populations (Greene et al. 2009).The use of haplotype-based association tests and the imputation of missing data can improve the power of GWAS. In the context of GWAS, there are several applications of missing data imputation. By means of a reference panel, imputation can be used to infer genotypes for ungenotyped known variants, to fill in the genotypes that have failed

to pass quality control standars, to combine results from many studies that have used different sets of variants and in family-based GWAS to infer the genotypes of unavailable family members (Dudbridge 2008). In addition, meta-analysis of multiple and smaller GWAS can provide adequate power to detect common variants with modest relative risk. Furthermore, the susceptibility variants reported from GWAS meta-analyses can be tested with smaller study samples. However, it should be noted that meta-analysis are only beneficial if the underlying genetic risk factors can be assumed to be shared across the different study populations used.

2.1.3 The Finnish population and its subisolates

Finland mainly became inhabited by two immigration waves: the first occurring about 4000 years ago coming from the East, and the second 2000 years ago from the South (Kittles et al. 1998). In the 1500's internal migrations caused a population explosion and small isolated villages were established by only a few founders throughout the wilderness (Figure 4) (Varilo and Peltonen 2004). The founding population of Finland was very small and has experienced multiple bottlenecks. Famines, wars and infectious diseases reduced the population size temporarily, caused losses of genetic variation and were followed by subsequent rapid expansions

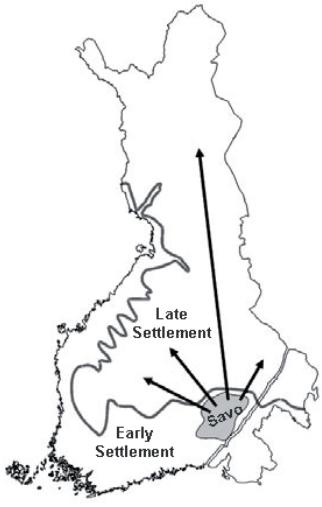

FIGURE 4. The settlement waves of Finland. Early habitation of Finland followed the coastline (Early Settlement). In the 16th century the internal migration movement originated from Southern Savo (shaded gray) to the central and eastern parts of Finland, resulting in genetically isolated subpopulations that were established by only a few founders and were isolated by distance from each other (Late settlement). Picture modified from Varilo et al 2003. Reproduced with permission of the copyright holder.

of the population size characterized by relative inbreeding and isolation, which has reduced further allelic diversity (Nevanlinna 1972; Norio 1966; Varilo and Peltonen 2004). Due to geographical, cultural and language barriers, the Finnish population has remained isolated from eastern and western neighbouring countries.

On the basis of Finnish population history and the special set of recessive diseases found in Finland, the Finnish population has been considered an isolated population (de la Chapelle 1993; Nevanlinna 1972; Norio et al. 1973; Varilo and Peltonen 2004). The Finnish genome shows decreased genetic diversity and increased LD compared to other populations in Europe and particularly in Africa (Lander and Schork 1994; Peltonen 2000). Accordingly, positional cloning has been most successful in indentifying the genetic variants of several rare monogenic disorders in Finland (Aittomaki et al. 1995; Cremers et al. 1990; Hastbacka et al. 1994; Kestilä et al. 1998; Peltonen et al. 1995; Savukoski et al. 1998) and it may be further advantageous in the identification of genetic variants in complex diseases (Service et al. 2006).

Due to the population history of Finland, the prevalences of several traits vary between subisolates and individuals categorized by the birthplaces of their grandparents show genetic clustering by region. A population substructure and geographically uneven distribution of disease alleles can be still detected (Jakkula et al. 2008; Salmela et al. 2006) (Figure 5). A subisolate population can be valuable for

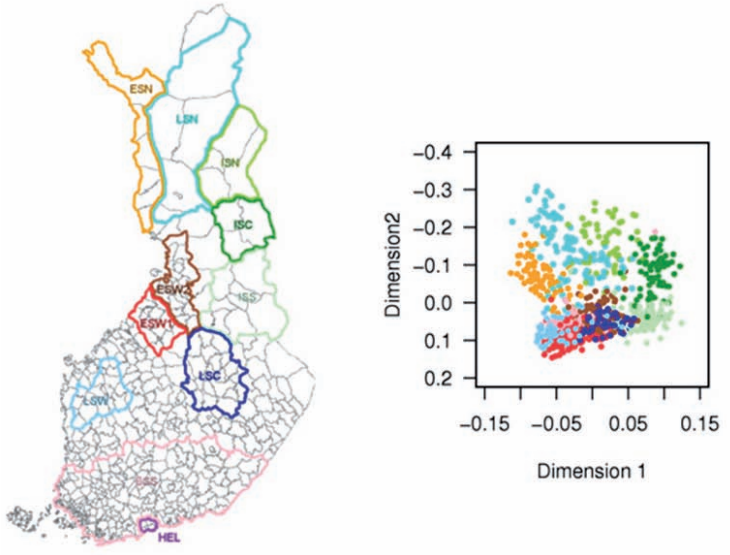

Figure 5. The subisolate population structure within Finland. Data of samples from ten distinct Finnish early- and late-settlement subisolates are shown with multidimensional scaling. The colored dots indicate samples from the corresponding colored regions of the Finland. Modified from Jakkula et al. 2008. Reproduced with permission of the copyright holder.

the genetic mapping and identification of rare penetrant risk variants. However, the use of isolates can lead to false positive association due to population stratification if the cases and controls originate from genetically distinct subpopulations having distinct allele frequencies (Hirschhorn and Daly 2005). In order to avoid population stratification, study samples should be selected carefully or family-based study samples should be used.

2.2 Bipolar disorder

2.2.1 Diagnosis

BP or manic-depressive illness is characterized by episodes of depression, mania, hypomania or mixed states and euthymia. Other psychiatric symptoms, such as delusions and hallucinations may occur during episodes (Goodwin and Jamison 2007). BP has a lifetime pattern of, at least, one manic or hypomanic episode with depressive episodes. Hypomania is differentiated from mania by the lower degree of impairment in function, and the disturbances of mood and behaviour are not accompanied by hallucinations and delusions (WHO 1993). One episode lasts from one week to several months. BP may start with depression and it can take many depressive episodes before the first mania episode occurs (Akiskal 2005). The depressive episodes are usually more frequent than the manic episodes, although the course of illness varies among individuals (Goodwin and Jamison 2007). BP is typically recurrent. The age of onset is usually in late adolescence, around 20 years of age and over 90 % of the patients experience their first manic episode before the age of 35 (Rihmer and Angst 2005). There is an elevated tendency for co-morbidity with drug abuse, alcoholism, compulsive behaviour, attention-deficit/hyperactivity disorder (ADHD) and anxiety (Krishnan 2005; Mantere et al. 2006). Bipolar disorder is associated with high levels of morbidity and mortality with up to 15 % of patients eventually committing suicide (Goodwin and Jamison 2007).

The diagnosis of BP is based on the evaluation of signs and symptoms as described in established diagnostic criteria. The current diagnostic criteria are presented in the International Classification of Diseases, tenth revision (ICD-10) (WHO 1993) and the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association, fourth revision (DSM-IV). BP is divided into bipolar disorder type I (BP-I), type II (BP-II) and not otherwise specified (BP-NOS) (Table 2–5).

TABLE 2. DSM-IV criteria for Manic Episode.

A. A distinct period of abnormally and persistently elevated, expansive or irritable mood, lasting at least one week (or less if hospitalization is required).

B. During the period of mood disturbance, three (or more) of the following symptoms have persisted (four, if the mood is only irritable) and have been present to a significant degree:
1. inflated self-esteem or grandiosity
2. decreased need for sleep
3. more talkative than usual or pressure to keep talking
4. flights of ideas or subjective expereince that thoughts are racing
5. distractibility (i.e.attention too easily drawn to unimportant or irrelevant external stimulus)
6. increased involvement in goal-directed activities or psychomotor agitation
7. excessive involvement in pleasurable activities with a high potential for painful consequences.

C. The symptoms do not meet criteria for a Mixed Episode.

D. The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others or to necessitate hospitalisatio to prevent harm to self or others or there are psychotic features.

E. The symptoms are not due to the direct physiological effects of a substance (e.g. drug of abuse, a medication or other treatment) or a general medical condition.

Diagnosis of bipolar disorder according to the Diagnostic and statistical manual of mental disorders, Fourth Edition (DSM-IV), Text Revision (American Psychiatric Association 2000).

TABLE 3. DSM-IV criteria for Hypomanic Episode.

A. A distinct period of persistently elevated, expansive or irritable mood, lasting throughout at least four days that is clearly different from the usual nondepressed mood.

B. During the period of mood disturbance, three (or more) of the following symptoms have persisted (four, if the mood is only irritable) and have been present to a significant degree:
1. inflated self-esteem or grandiosity
2. decreased need for sleep
3. more talkative than usual or pressure to keep talking
4. flights of ideas or subjective expereince that thoughts are racing
5. distractibility (i.e.attention too easily drawn to unimportant or irrelevant external stimulus)
6. increased involvement in goal-directed activities or psychomotor agitation
7. excessive involvement in pleasurable activities with a high potential for painful consequences.

C. The episode is associated with an unequivocal change in functioning that is uncharacteristic of the person when not symptomatic.

D. The disturbance in mood and the change in functioning are observable by others.

E.	The episode is not severe enough to cause marked impairment in social or occupational functioning or to necessitate hospitalisation and there are no psychotic features.
F.	The symptoms are not due to the direct physiological effects of a substance (e.g. drug of abuse, a medication or other treatment) or a general medical condition.

Diagnosis of bipolar disorder according to the Diagnostic and statistical manual of mental disorders, Fourth Edition (DSM-IV), Text Revision (American Psychiatric Association 2000).

TABLE 4. DSM-IV criteria for Major Depressive Episode.

A.	Five (or more) of the following symptoms have been present during the same two week period and represent a change from previous functioning; at least one of the symptoms is either depressed mood or loss of interest or pleasure: 1. depressed mood most of the day, nearly every day 2. markedly diminshed interest or pleasure, in all, or almost all, activies most of the day, nearly every day 3. significant weight loss or gain or decrease, or increase inappetite nearly every day 4. insomnia or hypersomnia nearly every day 5. psychomotor agitation or retardation nearly every day (observable by others) 6. fatigue or loss of energy nearly every day 7. feelings of worthless or excessive or inappropriate guilt nearly every day 8. diminshed ability to think or concentrate, or indecisiveness, nearly every day 9. recurrent thoughts of death, recurrent suicidal ideation without specific plan or a suicide attempt or a specific plan for committing suicide
B.	The symptoms do not meet criteria for a Mixed Episode.
C.	The symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning.
D.	The symptoms are not due to the direct physiological effects of a substance (e.g. drug of abuse, a medication or other treatment) or a general medical condition.
E.	The symptoms are not better accounted for by bereavement.

Diagnosis of bipolar disorder according to the Diagnostic and statistical manual of mental disorders, Fourth Edition (DSM-IV), Text Revision (American Psychiatric Association 2000).

TABLE 5. DSM-IV criteria for Mixed Episode.

A.	The criteria are met for both a manic Episode and for a Major Depressive Episode (except duration) nearly every day during at least a one week.
B.	The mood disturbance is sufficiently severe to cause marked impairment in occupational functioning or in usual social activities or relationships with others or to necessitate hospitalisation to prevent harm to self or others or there are psychotic features.
C.	The symptoms are not due to the direct physiological effects of a substance (e.g. drug of abuse, a medication or other treatment) or a general medical condition.

Diagnosis of bipolar disorder according to the Diagnostic and statistical manual of mental disorders, Fourth Edition (DSM-IV), Text Revision (American Psychiatric Association 2000).

*Bipolar disorder type I (*BP-I) is characterized by the occurrence of one or more manic or mixed episodes and depressive episodes. The predominant mood of manic episodes is elevated or irritable, which is accompanied by increased energy and activity, inflated self-esteem with grandiose ideas, pressure to talk, decreased need for sleep, increased sexual activity and impulsive behaviour. Mania may include psychotic symptoms as well, such as delusions and hallucinations. The most severe forms of mania cause marked impairment in social or occupational functioning requiring hospitalization. Manic episodes have to last at least one week in order to be diagnosed as BP-I. The predominant mood of the depressive episodes is a depressed mood and loss of interest, which is accompanied by reduced self-confidence, decreased energy and activity and feelings of guilt and worthlessness. The severe depression causes marked impairment in social or occupational functioning (American Psychiatric Association 1994; WHO 1993).

*Bipolar disorder type II (*BP-II) involves depressive and hypomanic episodes, but not manic episodes. Hypomanic episodes are a milder form of mania characterized by a persistent mild elevation of mood, increased energy, self-esteem, sociability and sexual energy. Furthermore, irritability and decreased need for sleep may be present in hypomania. Psychotic symptoms do not accompany hypomania. Hypomania is not severe enough to cause marked impairment in social or occupational functioning and it does not require hospitalization (American Psychiatric Association 1994; WHO 1993)

Cyclothymia has features similar to manic episodes and major depressive episodes without marked impairment in social or occupational functioning during hypomanic episodes. It is characterized by numerous short periods of mood swings, which are milder than in BP-I or BP-II, separated by short periods of normal mood. Cyclothymia is a chronic condition that periods without hypomanic or depressive symptoms less than two months at a time (American Psychiatric Association 1994; WHO 1993).

Mixed episode is a period in which both manic and major depressive episodes are present nearly every day over at least one week. In addition, it can be characterized by the presence of psychotic features. Mixed episodes cause marked impairment in social or occupational functioning and it may require hospitalization (American Psychiatric Association 1994; WHO 1993).

Rapid cycling mood is a period when manic and depressive moods change faster than is normal for the manic or major depressive phase (American Psychiatric Association 1994; WHO 1993).

Schizoaffective disorder (SA) shares features from both BP (mania and depression) and schizophrenia (SCZ) (hallucinations, delusions, disorganized speech, disorganized or catatonic behaviour and negative symptoms), thus falling in between SCZ and BP. SA can be specified as a bipolar type with concurrent or previous manic syndrome, or as a depressive type with the absence of current or

previous manic syndrome. All symptoms of BP and SCZ can appear together or independently (American Psychiatric Association 1994; WHO 1993).

Bipolar disorder, Not Otherwise Specified (NOS) is a diagnosis for those who show clear symptoms of BP, but do not meet the full diagnostic criteria. For example, hypomania without depression belongs to this group (American Psychiatric Association 1994; WHO 1993).

Seasonal affective disorder (SAD) is diagnose with a characteristic seasonal variation (American Psychiatric Association 1994; WHO 1993).

2.2.2 Epidemiology

Epidemiology is the study of the distributions of diseases in human populations and the variation of these distributions among different population subgroups. Prevalence is an epidemiological measure of the proportion of individuals that have a disorder at a specified time or during a specified period. Lifetime prevalence is the proportion of individuals in a population on a given day that have, at some point, been diagnosed for the disorder. As a result of differences in the methodologies and diagnostic classifications, measures of estimated lifetime prevalence of BP range from 0.1 to 4.8 % globally (Table 6) (Rihmer and Angst 2005). The World Health Organization (WHO) has assessed that BP is a marked global burden in society on par with SCZ in terms of premature deaths, leave of absence and poor health-related quality of life (Vos and Mathers 2000).

The annual incidence varies from 3 to 10 persons per 100 000 in the general population (Rihmer and Angst 2005). In Finland, the incidence of first-time hospital admissions for BP in 1994was reported at 12 persons per 100 000 (Rasanen P 1998), in contrast to the incidence of a first-time hospital admission for mania in the northern part of Finland that was reported at 1.7 persons per 100 000 per year (Veijola et al. 1996). The most recent Finnish population studies are based on the Health 2000 Study. They have estimated the lifetime prevalence of bipolar I disorder at 0.24 % in persons 30 years and older (Perala et al. 2007), and in young Finnish adults aged 19 to 34 years the prevalence is estimated to be 0.53 % for bipolar I disorder, 0.72 % for bipolar II disorder and 0.61 % for bipolar disorder, not otherwise specified (Suvisaari et al. 2009)

Many studies have reported an association between BP and stressful life events, which may also lengthen recovery periods (Altman et al. 2006; Hillegers et al. 2004). In addition, other mediating factors, such as personality traits, social support and sleep, may play role in increasing the risk of BP recurrence. Furthermore, pregnancy and childbirth may predispose to BP. It has been suggested that women have more than two–fold risk for BP after childbirth (Altman et al. 2006; Hillegers et al. 2004). Other predictors of bipolar episodes include: an increased number of previous episodes, decreased intervals between episodes, and persistence of

affective symptoms and episodes. However, evidence is still inconclusive and many aspects of the interaction between these factors and BP need to be considered in future studies (Tsuchiya et al. 2003).

Family-, twin- and adoption studies can be used to make a distinction between genetic and environmental factors contributing to disease susceptibility. Family studies have shown that BP aggregates in families. The morbid risk of developing BP for first-degree relatives of bipolar patients ranges from 1.5 to 17.9 % with a weighted average of 7.8 % (McGuffin and Katz 1989). Similarly, Smoller and Finn reported a weighted average of 8.7 % morbid risk to relatives of BP-I or -II probands (Smoller and Finn 2003). Relatives of BP probands have elevated morbid risk for other psychiatric diagnoses, such as unipolar disorder, schizoaffective disorder, cyclothymic personality and suicide (McGuffin and Katz 1989).

The difference between monozygotic (MZ) and dizygotic (DZ) concordance for a certain disorder indicates the genetic effect of the disorder. For complex traits, the MZ concordance is usually less than 100 % indicating that environmental factors contribute to disease risk. Twin studies have shown higher BP concordance in MZ twins than in DZ twins. Proband concordance rates have varied from 33 to 75 % for MZ twins and from 0 to 13 % for DZ twins. The results suggest a strong genetic component to BP, although its strength estimate varies between studies. In Finnish twin studies, the proband concordance rates were 43 % for MZ twins and 6 % for DZ twins, and heritability was estimated at 93 % (Kieseppa et al. 2004).

Adoption studies can determine the concordance risk for non-biological relatives of an affected individual and the proportional effects of genetic and environmental (post-natal) factors can be monitored. A study of 29 adoptees having BP reported more mood disorders among biological parents (31 %) than among adoptive parents (12 %) (Mendlewicz and Rainer 1977). However, the small number of study subjects and contradicting results limit the explanatory power of the adoption study results. It can be reasoned on the basis of these studies that the susceptibility to BP is largely genetic but environmental factors are needed to trigger the disorder, and thus BP is a result of a complex interaction between genes and environmental factors (Goodwin and Jamison 2007).

TABLE 6. Epidemiology of bipolar disorder.

	Caucasian population[a]	Finnish population[b]
Recurrence risk in sibling of a proband (λs)	5–10	-
Probandwise MZ twin concordance (%)	36–80	43
Proband DZ twin concordance (%)	0–13	6
Heritability estimate (%)	79–99	93

[a] Estimates from Craddock and Forty 2006.
[b] Estimates from Kieseppä et al. 2004.

2.2.3 Etiology and pathophysiology

Various abnormalities have been reported in BP patients, but little is known about the specific pathophysiology of bipolar disorder. The discovery of lithium's efficacy as a mood-stabilizer has led investigators to examine the signal transduction pathways involved in bipolar disorder. Lithium is a simple monovalent cation actively transported by the sodium-potassium-ATP pump through the cell membrane. The precise mechanism of action of lithium as a mood stabilizing agent is still unknown, but it has been found to play a role in several second messenger systems, such as cAMP and phosphoinositol pathways, by inhibiting the enzyme inositol monophosphate, leading to high levels of inositol triphosphate (Einat et al. 1998). Lithium is used for in the acute manic phase and as prophylaxis for recurrent manic and depressive episodes (Machado-Vieira et al. 2009). However, increasing evidence suggests that a significant number of patients do not respond adequately or cannot tolerate lithium side effects. It is hoped that by understanding lithium's therapeutic target, novel drugs with lithium-mimetic properties will be developed (Quiroz et al. 2004a; Quiroz et al. 2004b). Chronic administration of lithium and other mood stabilizers, such as carbamazepine, sodium valproate and lamotrigine, has been found to downregulate the arachidonic acid cascade in brain phospholipids, the formation of prostaglandin E(2), and/or the expression of arachidonic acid cascade enzymes that corresponds to the inhibition of arachidonic acid neurotransmission via dopaminergic- and glutamatergic N-methyl-D-aspartate receptors (Rapoport et al. 2009; Shi et al. 2008b).

In addition to lithium, typical antipsychotic drugs, such as dopamine and serotonin antagonists, are useful in the treatment of acute mania and as a maintenance treatment. The continued effect of these drugs on the dopaminergic (Brugue and Vieta 2007; Dunlop and Nemeroff 2007; Gershon et al. 2007; Nutt 2006), serotonergic and noradrenergic (Coppen 1969; Schildkraut 1965) systems has led to propositions of the roles of these systems in the aetiology of BP. Dopamine antagonists are not particularly selective and tend to block D_2 receptors in the dopamine pathways of the brain. Blocking dopamine in the receptors of the mesolimbic pathway has been thought to control psychotic experiences. Current evidence of the deficiencies of monoamines 5-hydroxytryptamine (5-HT, serotonin) and norepinephrine in the pathophysiology of bipolar disorder includes brain images studies (Cannon et al. 2007; Zipursky 2007), transgenic mouse models (Lesch and Mossner 2006; Urani et al. 2005) genetic association studies (Craddock and Forty 2006; Craddock and Jones 2001a; Kato 2007; Levinson 2005), and gene expression studies (Elashoff et al. 2007; Higgs et al. 2006). However, not all studies have supported their role in BP (Craddock and Forty 2006; Hayden and Nurnberger 2006).

Evidence for GABAergic and glutamatergic deficits in the pathophysiology and treatment of BP include neuroimaging, gene expression, gene association and

animal studies (Brambilla et al. 2003; Krystal et al. 2002; Kugaya and Sanacora 2005; Paul and Skolnick 2003; Sanacora 2008). Other neurotransmission systems, such as the cholinergic system, may also be involved in the pathophysiology of BP (Belzung et al. 2006; Berton and Nestler 2006; Hauger et al. 2006; Young 2006). Studies on the serotonergic systems of the CNS and the interaction among neurotransmitter receptors, G-proteins and signal transduction pathways, are required to better understand the pathology of BP.

There is robust evidence demonstrating the role of abnormalities of the hypothalamic-pituitary-adrenal axis in bipolar disorder. Abnormalities of various hormones and their regulatory systems, such as cortisol, thyroid-stimulating hormone and melatonin, may be central to the pathogenesis of BP (Takahashi et al. 2009). Hyperthyroidism has been associated with bipolar disorder, particularly with rapid cycling BP with predominant depressive episodes (Cassidy et al. 2002).

Technological improvements have made possible the study of the relationship between BP and neuropathology. Structural neuroimaging can be attained with tomography and magnetic resonance imaging (MRI), and functional information of the brain with functional MRI (fMRI). Studies of structural brain changes in BP have had highly variable reported findings and no consistent alterations have been found. Several studies have found abnormalities in the frontal lobe suggesting that the frontal lobe, limbic and basal ganglia circuits may play a role in the pathology of BP. MRI studies have reported an increased number of white matter hyperintensities in the frontal lobes, enlarged lateral ventricles, decreased overall brain grey matter volumes, the involvement of the temporal lobe; particularly hippocampus and amygdala, increased striatal size and decreased prefrontal cortex size (Davis et al. 2004; Lopez-Larson et al. 2002; McDonald et al. 2004). fMRI studies have shown abnormalities in the regulation of prefrontal-subcortical circuits (Chang et al. 2004), limbic-paralimbic blood flow and limbic metabolic alterations (Mayberg et al. 1999). These findings are also supported by magnetic resonance spectroscopy studies that implicate the presence of abnormal brain energy metabolism in BP (Dager et al. 2008).

Disruptions in biological rhythms have long been associated with BP (Jones 2001; Mansour et al. 2005; McClung 2007; Wehr et al. 1983). Abnormal sleep-wake, appetite and social rhythms are core symptoms of both manic and depressive episodes of BP. Sleep disturbance in BP has been hypothesized to be caused by abnormal circadian function, such as dysfunctional timing of sleep or the changed amount of sleep. Sleep disturbances may further trigger emotion dysregulation or vice versa (Dahl 2004; Harvey 2008; Harvey et al. 2006). In addition, severe social rhythm disruptions, such as moving, losing a job or returning from international trips, were found to be associated with onsets of mania in BP patients (Malkoff-Schwartz et al. 1998; Malkoff-Schwartz et al. 2000). Some treatments for BP seem to affect circadian rhythms by shifting, resetting and stabilizating these rhythms (McClung 2007).

Patients with BP present impaired cognitive performance compared to healthy individuals. Sustained attention, executive functioning, verbal learning and memory, verbal working memory and psychomotor speed are particularly impaired in bipolar patients in all phases of the disorder compared to healthy individuals (Glahn et al. 2004; Schneider et al. 2008). Impaired cognitive performance in verbal learning and memory, executive functions and attention may persist in remitted or euthymic patients (Martinez-Aran et al. 2009). These cognitive functions seem to worsen with illness progression (Kapczinski et al. 2008) and have a significant impact on general functioning. Cognitive impairments can be seen not only in bipolar patients but also in unaffected first-degree family members showing impairment in psychomotor performance speed and slight impairment in executive function. However, unaffected first-degree family members have normal performance in immediate memory, verbal fluency, and probably general intelligence (Robinson et al. 2006).

2.2.4 Overlap with related psychiatric disorders

BP and SCZ are recognized as separate disease entities with specific diagnostic criteria, but they share several clinical features and cognitive dysfunctions. Both disorders are highly heritable and are characterized by young ages at onset and a lifelong course. The disorders may include psychotic symptoms, but psychotic features are not always present in BP and predispose patients to increased risk for suicide (Berrettini 2003; Craddock et al. 2006; Moller 2003).

Co-segregation in families of BP with SCZ has been reported in many studies (Berrettini 2003; Maier et al. 1993; Weissman et al. 1984). Moreover, psychotic symptoms like hallucinations and delusions have been found to aggregate in bipolar pedigrees (Potash et al. 2001). As many as 50 % of BP patients have been reported to experience at least one psychotic episode in their lifetime (Dunayevich and Keck 2000). Psychotic symptoms usually occur during manic episodes (Berrettini 2003) and anti-psychotic drugs have been successful in decreasing the symptoms of both BP and SCZ (Tohen et al. 2003a; Tohen et al. 2003b; Tohen et al. 2003c). Many individuals with a severe psychiatric disorder have both mood and psychotic features falling between the diagnostic criteria of BP and SCZ, and thus are often diagnosed with schizoaffective disorder. This suggests that there may not be a neat biological distinction between these two disease entities.

Although bipolar and unipolar (UPD) disorders are not completely distinct disease entities, their distinction for the purposes of diagnosis and research is supported by evidence from their clinical features, epidemiological studies and treatment (Farmer and McGuffin 1989; Kendell 1987). In contrast to BP, UPD is more common, women have two-fold the prevalence of men and age of onset is later than in BP. Family studies have provided evidence of familial aggregation

of UPD and the results of twin and adoption studies suggest a moderate genetic component in UPD, having a heritability estimate of 33-42 %. Co-segregation in families of BP with UPD has been reported in many studies (Berrettini 2003; Maier et al. 1993; Weissman et al. 1984) showing a significantly higher prevalence of UPD in BP families (Joyce et al. 2004). However, according to the concept of continuity in mood disorders mania, hypomania, depression, mixed states and mood temperaments are variants of the same disorder, and thus they should not be seen as dichotomized traits.

2.3 Genetics of bipolar disorder

The evidence from family and twin studies for the involvement of genes in the etiology of bipolar disorder is based on investigations of several thousands of individuals and the heritability seems to be stronger than for many other complex diseases. Nonetheless, finding genes underlying the genetic and neurobiological background of BP has proven to be challenging and they remain elusive. Strategies for pinpointing risk genes for BP include linkage and association approaches.

2.3.1 Genome-wide linkage studies in BP

Several genome-wide scans have been conducted since the 1990s on a variety of sample sets of BP pedigrees, ranging from large densely affected pedigrees in isolates to large numbers of affected sibling pairs. Significant linkage findings on several chromosomal regions have been repeatedly implicated (Table 7). The strongly supported regions are 6q16–q22, 8q12q23–q24, 13q32–q34, 16p12–p13, 22q11–q22 and Xq24–26 (Baron 2002; Craddock and Jones 1999; Craddock et al. 2005; Ekholm et al. 2003; Maziade et al. 2005). Conflicting results of the linkage findings have been attributed to genetic and phenotypic heterogeneity that increase the rates of both false-positive and -negative findings as well as lead to reduced statistical power. It is unclear which phenotype best captures the underlying mechanism of BP. Different diagnostic categories (narrowly defined BP, intermediate or broad spectrum of BP diagnostic categories) are used in linkage studies, and therefore many linkage findings may refer to different phenotypic subtypes of BP.

TABLE 7. Suggestive linkage findings in bipolar disorder (Caucasian population).

Chromosome	Region
1	p36.33, p36.11, p32.1, p31.1, q23.3, q31.3, q32.1, q42.13, q42.2, q44
2	p25.1, p13.2, q11.2, q12.1, q14.1, q21.3, q24.1, q24.3, q31.1, q37.1, q37.3
3	p23, p21.32, p14.2, q23, q25.1, q26.31, q27.1, q28, q29
4	p16.1, p14, q31.23, q35.1, q35.2
5	p15.33, q32, q33.2, q33.3
6	p21.1, p23, q21, q23.3
7	q22.1, q32.1, q36.1
8	p21.1, p12, q13.1, q13.2, q24.13, q24.22, q24.23, q24.3
9	p22.3–21.1, q21.13, q21.32, q31.1, q32,
10	p12.33, q11.21–22.1, q21.3, q26.12, q26.2
11	p15.5, q12.2
12	q13.12, q21.1, q23.1, q24.31, q24.32
13	q12.11, q12.3, q13.2, q14.2, q32.1, q32.2, q32.3, q33.3
14	q24.1, q24.1–32.12, q22.2, q22.3, q32.33
15	q22.2, q26.2, q26.3
16	p13.3, p13.2. p13.13, p12.1, q12.2, q23.1
17	p12, q11.2, q25.3
18	p11.1–q12.3, p11.32, p11.31, p11.23, p11.22, p11.21, q12.1, q22.1. q22.3, q23
19	p13.3, q13.33
20	p13, p12.2, q13.13, q13.33
21	q21.1, q22.2, q22.13, q22.3
22	q11.1, q12.3
X	p22.11, q22.3, q26.1, q27–28

Three meta-analyses of BP linkage have been published. The aim of the meta-analyses was to identify and produce a more general understanding of the genetic predisposition to BP. Badner and Gershon examined seven published genome-wide scans conducted worldwide (Badner and Gershon 2002). They established significant linkage to BP on chromosomes 13q and 22q. Similarly, Segurado et al. examined 18 genome-wide scans (Segurado et al. 2003). However, they did not find significant evidence for linkage in any of the analyses and there were no overlapping results with the previous meta-analysis study by Badner and Gershon.

The study by Segurado et al. provided only moderate support for chromosomal regions 9p22.3–21.1, 10q11.21–22.1, 14q24.1–31.12 and 18p–q. McQueen et al. conducted a combined analysis of 11 bipolar genome-wide scans by using original genome-scan data rather than summary statistics, as were used in the two previous meta-analyses. They found significant evidence for two susceptibility loci on chromosomes 6q and 8q (McQueen et al. 2005). Therefore, although meta-analyses provide some support for the genetic findings for BP, the genetic heterogeneity among different populations and samples is relatively high.

In the first Finnish linkage study of BP, microsatellite markers were analyzed on chromosome X. Linkage was found in an extended BP family and locus Xq24–27.1 (LOD = 3.54) (Pekkarinen et al. 1995). On the locus Xq24–27.1 a distinct haplotype across a 19 centimorgan (cM) region was found to co-segregate with BP. Since new individuals from the extended family were added and diagnoses were updated, the extended family was re-analyzed separately from the other BP families (n = 40) in the fine-mapping study (Ekholm et al. 2002). When testing all 41 families together, a LOD score of 2.78 was observed at Xq28. However, when the extended pedigree was excluded from the analysis, a LOD score of 1.34 was observed. The multipoint analysis gave a LOD score of 4.5 when only the extended family was analyzed, suggesting that a relatively rare gene predisposing to BP is enriched in the extended family (Ekholm et al. 2002).

A genome-wide linkage scan with 389 microsatellite markers was conducted in 41 Finnish BP families. Three loci, 4q32, 12q23 and Xq25 produced LOD scores > 2.0 and one locus, 16p12, a LOD score >3.0 in two-point analyses. Loci 4q32, 12q23 and 16p12 were fine-mapped with additional unaffected individuals from the 41 BP families. An X-linked extended pedigree was systematically analyzed separately from the other families. In a three-point analysis, locus 4q32 (LOD = 3.6) and 16p12 (LOD = 2.7) provided significant evidence of linkage (Ekholm et al. 2003). This genome-wide scan was analyzed together in a meta-analysis with 17 other genome-wide scans of BP conducted worldwide. Significant linkage was not reached in the analyses. However, some evidence of linkage on regions 8q, 9p–q, 10q, 14q and 18p–q was observed in the meta-analysis (Segurado et al. 2003).

2.3.2 Candidate gene studies in BP

Two main approaches have been employed to identify genes in BP: the positional candidate gene approach, in which genes located under linkage peaks are selected for study; and the functional candidate gene approach, in which genes are selected based on their involvement in the biological processes implicated in the disease. Most BP functional candidate gene studies have focused on neurophysiologic alterations associated with the disease in animal models or pharmacological studies (Craddock and Jones 1999; Craddock and Jones 2001b). Both positive and

negative associations have been reported for candidate genes (Table 8), as seen in the association studies of other psychiatric disorders.

The imbalance or defective function of the monaminergic neurotransmitter system is related to the etiopathogenesis of BP. Consequently, some of the most widely investigated genes in BP have been those related to amine metabolism, such as catechol-O-methyltransferase (*COMT*) on 22q11.21 and monoamine oxidase A (*MAOA*) on Xp11.3. *COMT* encodes for an enzyme that catalyzes the degration of catecholamines, including dopamine, epinephrine and norepinephrine; while *MAOA* encodes a protein that oxidizes neurotransmitters, such as serotonin, dopamine and norepinephrine. To date, results for both genes have been controversial with positive association findings along with perhaps an even greater number of negative replications (Abdolmaleky et al. 2006; Anguelova et al. 2003; Lasky-Su et al. 2005b; Prata et al. 2006; Preisig et al. 2000; Serretti et al. 2006). Variants within *COMT* have been found to be associated with BP (Chen et al. 2004; Shifman et al. 2004), and in addition with various cognitive functions (Egan et al. 2001; Ehlis et al. 2007; Goldberg et al. 2003; Harris et al. 2005) in several samples, including one from the Finnish population (Soronen et al. 2008). A CA-repeat microsatellite in intron 2 has been consistently associated with BP in both Caucasian and Asian populations (Furlong et al. 1999; Lin et al. 2000). Another variant in the promoter of *MAOA* was found not associated in BP in Asian and Caucasian populations, except in one study on five pooled samples (Preisig et al. 2000). One study in Caucasians has recently reported association with BP of the haplotype composing of both the CA-repeat microsatellite and the promoter variant (Muller et al. 2007).

Serotonin transporter (*SLC6A4*) and neuronal tryptophan hydroxylase (*TPH2)* have also been widely studied in BP. The *SLC6A4* gene is located on 17q11.1–q12, which is has been positively linked to BP in two independent studies (Murphy et al. 2000; Tomas et al. 2006). A 48 base-pair promoter polymorphism has been associated with BP and four meta-analyses confirmed the finding (Anguelova et al. 2003; Cho et al. 2005; Furlong et al. 1998; Lasky-Su et al. 2005a). The *TPH2* gene on 12q21.1 encodes a rate-limiting enzyme in the biosynthesis of serotonin in the central nervous system (Walther and Bader 2003). Variants within the gene have consistently been associated with BP in seven independent studies (Cichon et al. 2008; Grigoroiu-Serbanescu et al. 2008; Harvey et al. 2007; Harvey et al. 2004; Lopez et al. 2007; Roche and McKeon 2009; Van Den Bogaert et al. 2006). However, two studies have reported negative findings (De Luca et al. 2004; Mann et al. 2008).

The D-amino acid oxidase activator *DAOA (G72 /G30*) locus is located on chromosome 13q22–34 where genes G72 and G30 overlap, but are transcribed in opposite directions. *DAOA* is a primate-specific gene encoding a mitochondrial protein that promotes mitochondrial fragmentation and dendritic branching. It is expressed in the central nervous system, in amygdala and caudate in particular (Craddock and Forty 2006). *DAOA* is involved in glutamate signalling playing a

role in the activation of N-methyl-D-aspartate receptors (Chumakov et al. 2002; Williams et al. 2006), which have been shown to have multiple effects on learning and memory. There have been at least five positive family-based association reports in two US BP family samples, which were replicated in another US family sample and two BP case-control association studies using German and UK samples (Chen et al. 2004; Hattori et al. 2003; Schulze et al. 2005; Schumacher et al. 2004; Williams et al. 2006). The Finnish bipolar family study sample showed marginal evidence for association of variants from DAOA with bipolar spectrum disorder (Soronen et al. 2008), but a significant association with visuospatial ability. Interestingly, the same variant (Arg30Lys) associated in another study with verbal memory function in schizophrenia (Donohoe et al. 2007)..

The brain derived neurotrophic factor (*BDNF*) gene is a member of the neurotrophin superfamily. This gene plays an important role in promoting and modifying growth; the development and survival of neuronal cell populations; and the mature central nervous system. It is also involved in activity-dependent neuronal plasticity and connectivity in the central nervous system. The change in the function and structure of these processes may be induced by stress and antidepressants, and therefore *BDNF* could play an important role in mood disorders. The *BDNF* gene is located on chromosome 11p13, which has been implicated in some linkage analyses of BP (Green and Craddock 2003). This gene and particularly the Val66Met variant have consistently associated with BP and memory impairments (Martinez-Aran et al. 2004; Rybakowski et al. 2003; Rybakowski et al. 2006; Tramontina et al. 2009) in a total of eleven studies of mainly Caucasians (Geller et al. 2004; Green et al. 2006; Lohoff et al. 2005b; Neves-Pereira et al. 2002; Schumacher et al. 2005; Sklar et al. 2002; Strauss et al. 2004). There have also been positive family-based association reports in BP samples of European-American origin (Kremeyer et al. 2006). These studies have shown the over-transmission of the common Val allele and there is also evidence of associated multilocus haplotypes in this gene. Controversely, six association studies in Caucasian, Japanese and Chinese populations have reported negative evidence for association (Green et al. 2006; Lohoff et al. 2005a; Nakata et al. 2003; Oswald et al. 2004; Skibinska et al. 2004).

The purinergic receptor ligand-gated ion-channel 7 gene (*P2RX7*) encodes the P2X7 receptor, which is a non-specific cation channel that is activated by high levels of ATP. It has a role in neurotransmitter release and inflammatory responses. *P2RX7* is located on 12q24, which has been previously implicated by several linkage studies of BP (Curtis et al. 2003; Dawson et al. 1995; Ekholm et al. 2003; Ewald et al. 1998; Maziade et al. 2001; Morissette et al. 1999; Shink et al. 2005) and UPD (Abkevich et al. 2003; McGuffin et al. 2005; Zubenko et al. 2003). More recently, a non-synonymous variant within *P2RX7* has been reported to associate with BP in two independent studies (Barden et al. 2006; McQuillin et al. 2009) and with UPD (Hejjas et al. 2009; Lucae et al. 2006). Nonetheless, Green et al. studied 1,723 cases of BP and UPD and 1,204 controls from the UK population as well as sequenced

two BP families that previously showed linkage to the 12q23–24 region, and they failed to find evidence to support the association of *P2RX7* with BP or UPD (Green et al. 2009).

The Disrupted-in-Schizophrenia-1 gene (*DISC1*) encodes a multifunctional protein that plays important roles in neurodevelopment and synaptic modulations by interacting with several proteins. It is highly expressed in the central nervous system, particularly in the hippocampus and the cerebral cortex (Austin et al. 2003; Ozeki et al. 2004; Schurov et al. 2004). The genomic locus was originally identified and linked with SCZ and related psychiatric disorders in a large Scottish pedigree. Members of that large family carried a balanced (1;11) (q42.1;q14.3) translocation that segregated with major psychiatric disorders, including SCZ, BP and recurrent major depression. The translocation disrupts two genes, *DISC1* and *DISC2*, at 1q42 (Blackwood et al. 2001; Millar et al. 2000a; Millar et al. 2000b). In recent years, the evidence for linkage and association of BP with *DISC1* has been accumulating (Curtis et al. 2003; Detera-Wadleigh et al. 1999; Gejman et al. 1993; Hodgkinson et al. 2004; Macgregor et al. 2004; Maeda et al. 2006; Thomson et al. 2005). Overtransmission of a risk haplotype to affected BP females, who also showed lower levels of *DISC1* messenger RNA (mRNA) expression in their lymphoblast's, was reported in a recent family-based association study (Maeda et al. 2006). A recent study revealed the presence of locus heterogeneity and the interplay of variants within DISC1 in the aetiology of major psychiatric disorders (Hennah and Porteous 2009).

The circadian locomotor output cycles kaput gene (*CLOCK)* appears to be involved in the regulation of the circadian rhythm. A variant in the 3′ flanking region of *CLOCK* (3111 T to C) has associated with a higher recurrence rate of bipolar episodes (Benedetti et al. 2003), greater insomnia and a decreased need for sleep in bipolar patients (Serretti et al. 2003; Serretti et al. 2005). In addition, other members of the molecular clock have been implicated in BP. Period homolog 3 (*PER 3*) and Aryl hydrocarbon receptor nuclear translocator-like (*ARNTL*) genes have associated with BP (Mansour et al. 2006; Nievergelt et al. 2006). Interestingly, *ARNTL* encodes the protein Arntl that dimerizes Clock protein and Clock-Arntl heterodimers further seem to drive the *PER 3* oscillations (Gekakis et al. 1998). Recently, haplotypes in *BMAL1* and *Per3* were found to significantly associate with BP (Nievergelt et al. 2006) and variants in *BMAL1* and in the *Timeless* gene have also positively associated with BP (Mansour et al. 2006).

One of the promising candidate genes in BP is the glycogen synthase kinase 3-β gene (GSK3β), which modulates the circadian clock and is the target of lithium (Gould and Manji 2005). GSK3β encodes glycogen synthase kinase 3B, a proline-directed serine-threonine kinase that participates in the wnt signalling pathway and is involved in energy metabolism, neuronal development and body pattern formation (Serretti and Mandelli 2008). This gene and particularly the T-50C variant have associated with a later age of onset of BP, total sleep deprivation and

chronic lithium treatment (Benedetti et al. 2004; Benedetti et al. 2005). To date, results for this gene have been controversial with positive association findings along with negative replications (Lee et al. 2006; Michelon et al. 2006; Szczepankiewicz et al. 2006a; Szczepankiewicz et al. 2006b). However, recent CNV studies of BP have reported increased duplication of this region (Lachman et al. 2007).

A large number of candidate genes, including functional and positional candidate genes have been evaluated. Several of these genes have been associated with the disorder in independent studies (including *BDNF, DAOA*, *DISC1, SLC6A4* and *TPH2*), but none of their explicit roles have been established. The clinical heterogeneity of BP and its phenotypic and genetic overlap with other psychiatric disorders including in particular schizophrenia, schizoaffective disorder and major depressive disorder, cause problems for genetic studies. Affected patients may not share the same underlying biology, and therefore the use of endophenotypes, such as neuropsychological performance, would be a more optimal phenotype definition for genetic studies.

TABLE 8.The most widely studied and associated genes with bipolar disorder.

Gene	Function	Location
ANK3[a]	Plays key roles in activities such as cell motility, activation, proliferation, contact and the maintenance of specialized membrane domains	10q21
ARNTL	Encodes a protein that forms a heterodimer with CLOCK	11p15
BDNF	Promotes the survival of neuronal populations	11p13
CACNA1C[a]	Encodes an alpha-1 subunit of a voltage-dependent calcium channel that mediate the influx of calcium ions into the cell upon membrane polarization	12p13
CLOCK	Encodes a protein that forms a heterodimer with ARNTL	4q12
COMT	Encodes catechol-O-methyl transferase; catalyses the O-methylation and inactivation of catecholamine neurotransmitters and catechol hormones	22q11
CREB1	Encodes a transcriptional activator that binds to the cAMP response element	2q33
DAOA	Encodes an activator of the FAD-dependent enzyme D-amino acid oxidase, which degrades the gliotransmitter D-serine, a potent activator of NMDA type glutamate receptors	13q22–34
DFNB31[a]	Interacts with a CASK, and may be involved in the formation of scaffolding protein complexes that facilitate synaptic transmission in the central nervous system	9q32–34
DGKH[a]	Encodes a type 2 diacylglycerol kinase (DGK) enzyme that is involved in regulating the intracellular concentrations of diacylglycerol and phosphatidic acid	13q14

Gene	Function	Location
DISC1	Encodes an integral component of the pericentriolar material	1q42
DPY19L3[a]	Encodes Dpy-19-like protein 3	19q13
GSK3β	Encodes glycogen synthase kinase 3β, a proline-directed serine-threonine kinase that participates in the wnt signalling pathway	3q13
HTR1A	Encodes the type 1A receptor for 5-HT	5q12
HTR1B	Encodes the type 1B receptor for 5-HT	6q14
HTR2A	Encodes the type 2A receptor for 5-HT	13q14
MAOA	Encodes the monoamine oxidase A enzyme that degrades amine neurotransmitters, such as dopamine, noradrenaline and 5-HT	Xp11
MYO5B[a]	Encodes a protein that may be involved in plasma membrane recycling	18q21
NAP5[a]	Interacts with the SH3-containing region of the adapter protein Nck	2q21
NTRK2[a]	Encodes a neurotrophic tyrosine receptor kinase (NTRK) that upon neurotrophin binding phosphorylates itself. NTRK is a member of the MAPK pathway	9q21
P2RX7	Encodes an ATP-gated cation channel	12q24
PALB2[a]	Binds to and colocalizes with the breast cancer 2 early onset protein (BRCA2) and permits the stable intranuclear localization and accumulation of BRCA2	16p12
PER3	Encodes a protein that plays a role in the circadian rhythms of locomotor activity, metabolism, and behavior	1p36
SLC642	Encodes an amine transporter and terminates the action of noradrelanine by its high-affinity sodium-dependent reuptake into presynaptic terminals	16q12
SLC6A4	Encodes an amine transporter and terminates the action of 5-HT by its high-affinity sodium-dependent reuptake into presynaptic terminals	17q11
TPH2	Encodes neuronal tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT	12q21

[a] Genes significantly associated in genome-wide association studies in bipolar disorder.

2.3.3 Genome-wide association studies in BP

In the last years, progress in the development of public databases, analysis methods and high-throughput genotyping technology has enabled large-scale genome-wide association tests with common, complex diseases. The GWAS approach has revealed many variants that show consistent association with complex disorders, such as type 2 diabetes (Saxena et al. 2007), Crohns's disease (Rioux et al. 2007), prostate cancer (Gudmundsson et al.2007) and schizophrenia (Shi et al. 2009; Stefansson et al. 2009). Recently, several groups have reported GWAS findings for bipolar disorder (Table 8) using either pooled (Baum et al. 2008) or individually genotyped samples (Baum et al. 2008; Ferreira et al. 2008; Scott et al. 2009; Smith et al. 2009; The Wellcome Trust Case Control Consortium 2007). The Wellcome Trust Case Control Consortium (WTCCC) studied 1,838 cases of BP and 2,938 controls from the UK using the Affymetrix 500K GeneChip (The Wellcome Trust Case Control Consortium 2007)t. This large study reported only one genome-wide significant association on 16p12 (rs420259, $P = 6.3 \times 10^{-8}$) that passed their stringent criteria for genome-wide significance ($P = 5 \times 10^{-7}$)(The Wellcome Trust Case Control Consortium 2007). Baum et al. revealed the most significant association with variants in the diacylglycerol kinase (*DGKH*) gene at 13q14.11 (rs1012053 in intron 1, $P = 1.5 \times 10^{-8}$) in two sample sets, one German (772 cases and 876 controls) and the other from the US (NIMH collection: 461 cases and 563 controls), by genotyping pooled DNA for 550K SNPs (Baum et al. 2008). In 1,461 cases and 2,008 controls from a US Caucasian population using the Affymetrix 500K GeneChip, Sklar et al. detected the strongest association with a variant in Myosin 5B (*MYO5B*) on 18q21.1 ($P = 1.7 \times 10^{-7}$), as well as association with tetraspanin-8 (*TSPAN8*) on 12q21.1 and epidermal growth factor receptor (*EGFR*) on 7p11.2, (Sklar et al. 2008).

There was no overlap of the most significant results between the studies of the WTCCC and Baum et al. when in-depth comparison was performed (Gershon et al. 2008), nor was there an overlap between the studies of Baum et al. and Sklar et al. (Sklar et al. 2008). However, when Sklar et al. compared their top 200 SNPs with the WTCCC results they observed a consistency with a variant in the alpha 1C subunit of the L-type voltage-gated calcium channel (*CACNA1C*) gene on 12p13.33 (Sklar et al. 2008). The lack of overlap of the most significant results among these three studies may be due to different study designs, genetic heterogeneity, or the fact that variants may have relatively modest effects on susceptibility to BP and are not easily detected.

In order to validate genome-wide significant association results, Ferreira et al. studied 4,387 cases of BP and 6,209 controls by combining the results of two previously studies, the WTCCC and Sklar et al. studies, and one new GWAS (1,098 cases of BP and 1,267 controls) using 1.8 million variants (Ferreira et al. 2008). This meta-analysis identified a region of strong association in ankyrin G (*ANK3*) on 10q21 (rs10994336, $P = 9.1 \times 10^{-9}$). In addition, they found further support

for the association in the *CACNA1C* region on 12p13 (rs1006737, OR = 1.18, $P = 7.0 \times 10^{-8}$).

Smith et al. conducted GWAS studies in two sample sets: one of European ancestry (n = 1,001 cases and n = 1,033 controls) and the other of African ancestry (n =345 cases and n = 670 controls). For the European ancestry sample, the strongest evidence for association maps to an intergenic region on Xq27.1 (rs5907577, $P = 1.6 \times 10^{-6}$) and the Nck-associated protein 5 gene (*NAP5*) on 2q21.2 (rs10193817, $P = 9.8 \times 10^{-6}$). For the African Ancestry sample, the strongest association was detected with a variant in the dpy-19-like 3 gene (*DPY19L3*) on 19q13.11 (rs2111504, $P = 1.5 \times 10^{-6}$) and the neurotrophic tyrosine kinase receptor type 2 gene (*NTRK2*) on 9q21.33 (rs2769605, $P = 4.5 \times 10^{-5}$). However, significant genome-wide association was not detected in either of the sample sets. Some support for the previous association in *ANK3* and *C15Orf53* at the 15q14 region was found (Smith et al. 2009). The most recent GWAS with 2,076 BP cases and 1,676 controls of European ancestry using the Illumina 550K chip and data for >2.3 million genotyped and imputed variants did not provide support for the previous BP GWAS findings. In addition, they performed a 3-study meta-analysis with the previously published (WTCCC) BP study (n = 3,683 cases and 14,507 controls), but there were no genome-wide significant associations (Scott et al. 2009).

GWAS results strongly suggest that BP is influenced by the effects of multiple rare variants rather than common variants that would be shared by many populations. The strong heritability of BP and the relative lack of the shared risk variants suggest that there are few, if any, common risk variants on bipolar disorder.

2.3.4 Copy number variation studies in BP

Recent analysis of copy number variations (CNVs) has shown that a considerable number of neuropsychiatric disorder candidate genes have their coding elements disrupted by polymorphic CNVs, which suggests they have a substantial contribution to disease. The first CNV study in BP revealed a CNV in the GSK3β locus on 3q13.3 with increased frequency in BP patients compared to controls ($P = 0.002$). The finding suggests that GSK3β may be involved in BP susceptibility in some individuals (Lachman et al. 2007). The systematic genome-wide CNV analysis of BP (n = 1,001 cases and 1,033 controls) showed that singleton deletions induce a 1.31-fold increased risk of BP and singleton deletions more than 100 kb were present in 16.2 % of BP cases in contrast to 12.3 % of controls ($P = 0.007$). The effect was even greater among patients with earlier onset of mania. These results suggest that very rare *de novo* deletion events increase risk of BP (Zhang et al. 2009). In addition, a recent meta-analysis of datasets gathered for the study of multiple psychiatric disorders showed an association of the microduplication of a 600 kb genomic region on 16p11.2 with schizophrenia ($P = 4.8 \times 10^{-7}$), bipolar disorder ($P = 0.017$) and autism ($P = 1.9 \times 10^{-7}$) (McCarthy et al. 2009).

3 Aims of the study

The aim of the present study was to investigate the genetic basis of BP and to identify susceptibility genes for BP and related cognitive features in Finnish families. The following specific aims of this study were:

1. To examine the role of the allelic variants of the *TSNAX/DISC1* gene cluster in psychotic and bipolar spectrum disorders and related cognitive endophenotypes.

2. To examine the role of the widely studied six candidate genes (*SLC6A4, TPH2, BDNF, CREB1, NTRK2* and *P2RX7*) from three seemingly distinct signalling systems in the genetic background of psychotic and bipolar spectrum disorders and related cognitive endophenotypes

3. To identify genomic susceptibility regions to bipolar disorder by investigating bipolar families originating from the late settlement region of Finland.

4. To elucidate the role of the allelic variants associating with bipolar disorder in the recent GWA studies.

4 Material and methods

4.1 Study sample (I, II, III and IV)

All Finnish families collected and ascertained for bipolar disorder were used in our study. The study sample consisted of 723 individuals including 298 affected with broad mood disorder in 179 families. 69 families altogether had two or more affected individuals with DNA samples available. The set of 179 families included 44 dizygotic twins (with broad mood disorder n = 25) from 29 twin pairs and 6 monozygotic twins (with broad mood disorder n = 6) from 6 twin pairs (Kieseppä et al. 2004). The diagnoses of individuals in 21 BP families of the 41 families included in previous bipolar genome-wide linkage scan analyses (Ekholm et al. 2003) were ascertained and updated in 2003 using the Structured Clinical Interview method (SCID) (Antila et al. 2007a; Spitzer et al. 1997). The new method led to 30 changes in diagnosis: 20 unknown cases were ascertained to have bipolar disorder or recurrent major depressive disorder (rMDD); and 10 cases of broad type bipolar disorder including BP-I, BP-II, bipolar disorder not otherwise specified (BP-NOS), cyclothymia and rMDD changed to schizophrenia or other non-bipolar psychoses.

For studies I and III, two alternative diagnostic categories were applied comprising a group of patients with bipolar spectrum disorders, i.e. bipolar disorder type I, II, BP-NOS and cyclothymia (n = 227), and a group of patients with psychotic disorders, i.e. bipolar disorder type I with intermittent psychotic features, psychotic depression, schizophrenia, schizoaffective disorder and psychosis NOS (n = 251). There were 162 individuals belonging to both groups. In study III, the set of 118 families (n = 543) having at least two affected individuals was selected as a subsample in Table 9). For study II and IV, a group of patients with broad mood disorder, i.e. bipolar disorder type I, II and not otherwise specified, manic type of schizoaffective psychosis, cyclothymia, and recurrent depression, was used for analysis. In study IV, patients with bipolar disorder type I (n = 214) were studied.

TABLE 9. The composition of the study sample available for analysis

	Total	Males	Females	Familial cases	Neuropsychologically assessed
Family sample[a]					
Bipolar disorder	214	110	104	171	63
Bipolar spectrum disorder[b,d]	227	118	109	173	66
Psychotic disorder[c,d]	251	134	117	212	67
Other mental disorders	46	19	27	45	26
Unaffected family members	361	153	208	241	51
Total	723	344	379	544	158
Control sample					
Anonymous trio sample	171	101	70	0	0

[a] The families were ascertained for BP through the nationwide register data. The family sample includes 44 dizygotic and 6 monozygotic twins.
[b] Includes cases with bipolar disorder type I (n = 214) , II (n = 5), not otherwise specified (n = 6) and cyclothymia (n = 2).
[c] Includes cases with bipolar disorder type I with intermittent psychotic features (n = 162), psychotic depression (n = 15), schizophrenia (n = 14), schizoaffective disorder (n = 51), and psychosis NOS (n = 9).
[d] Overlap of 162 affected individuals between the diagnostic categories.

For study II, we selected 23 late settlement families with at least two affected siblings. Families of the late settlement were chosen according to the birthplaces of the siblings' grandparents such that at least one grandparent from each family originated from the late settlement region of Finland. The late settlement families were selected for our various genetic analyses in distinct diseases, because the families originating from that region are, according to several genetic and population LD studies, genetically more homogenic than nation-wide samples (here the larger set of 179 families). The 23 families originating from the late settlement region consisted of 147 individuals from 21 nuclear families and two extended families. The largest extended family consisted of four generations with 18 affected subjects. The study sample included 68 affected individuals with a diagnosis of a broad mood disorder, i.e. bipolar disorder type I, II, or not otherwise specified, manic type of schizoaffective psychosis, cyclothymia, or recurrent depression (Table 10). The broad mood disorder model was used since unipolar disorder is the most common disorder in families with bipolar disorder. Of unipolar disorder patients, 12-41%

patients eventually develop mania or hypomania (Akiskal et al. 1995; Goldberg et al. 2001).

TABLE 10. The composition of the late settlement family sample of 23 families used for genome-wide linkage scan.

Family sample[a]	Total	Males	Females
Broad mood disorder[b]	68	39	29
Other mental disorder	21	11	10
Unaffected family members	58	24	34
Total	147	74	73
Anonymous trio sample	0	0	0

[a] The families were ascertained for bipolar I disorder through nationwide register data.
[b] Includes cases with bipolar disorder type I (n = 214), bipolar disorder type II (n = 5), bipolar disorder not otherwise specified (n = 6), manic type of schizoaffective psychosis (n = 46), cyclothymia (n = 2) and recurrent depression (n = 25).

A random sample of 57 control families from the Finnish population consisting of three family members (father, mother and one child) was combined with the whole family sample for the association analysis in order to account for the ascertainment bias of the family sample.

4.2 Examined phenotypes

4.2.1 Clinical diagnosis (I–IV)

The bipolar family sample was collected utilizing The National Hospital Discharge Register in order to identify individuals born between 1940 and 1969 and hospitalized with a diagnosis of BP between 1969 and 1991. The National Public Health Institute obtained permission from the Ministry of Social Affairs and Health to access data for the years 1968–1991 for genetic studies of mental disorders (Hovatta et al. 1997). The diagnoses in the Hospital Discharge Register were based on the International Classification of Diseases, version 8 (ICD-8) (WHO 1967) before 1987, and between 1987 and 1991 they were based on the Diagnostic and Statistical Manual of Mental Disorders, revised third edition (DSM-III-R) (American Psychiatric Association 1987). From the register, we identified all patients with at least one diagnosis of bipolar disorder (296.10 or 296.30 (ICD-8), or 296.4, 296.5 or 296.6 (DSM-III-R)).

Two psychiatrists assessed the diagnoses on the basis of the medical records, blind to each other and according to DSM-IV-criteria (American Psychiatric Association 1994). Diagnoses were discussed and, if needed, the opinion of a senior psychiatrist was obtained for the consensus. No disagreement remained. Only patients with bipolar disorder or the manic type of schizoaffective disorder were regarded as eligible probands. The latter diagnosis was accepted, because there is accumulating evidence that it might have a shared genetic background with bipolar I disorder (Kendler and Gardner 1998). The probands were then linked to family members by gathering information from the National Population Register. The Hospital Discharge Registry was used for the diagnosis of the probands' family members. In addition, twins with a diagnosis of BP were identified and collected in the same manner as the family sample from the Finnish twin cohorts (Kieseppa et al. 2004).

4.2.2 Neurocognitive variables (I, II)

During 1999–2004, 158 (study II: 159) family members from 57 (study II: 65) nuclear and extended bipolar families completed a comprehensive neuropsychological test battery (Antila et al. 2007a; Antila et al. 2007b).

The neuropsychological test battery, from which the cognitive traits were obtained, is a series of tests that uses well validated, internationally well-known neuropsychological measures to evaluate an individual's cognitive ability. The test includes Vocabulary, Similarities, Digit Symbol and Block Design subtests from the Wechsler Adult Intelligence Scale-Revised (WAIS-R)(Weschler 1981), which assess basic ability, abstraction, psychomotor processing speed and visuospatial ability, respectively. From the Wechsler Memory Scale-Revised (WMS-R)(Weschler 1987), we included: the Digit Span Forward and Backward tests, which measure auditory attention and verbal working memory, respectively; the Visual Span Forward and Backward tests, which assess visual attention and visual working memory, respectively; and the Logical Memory and Visual Reproduction subtests to assess verbal and visual memory, respectively, in both immediate and delayed conditions. The California Verbal Learning Test (CVLT) was included to assess (Delis DC 1987) the following variables: learning (total recall from trials 1-5), short-delay memory, long-delay memory, recognition memory, retention and perseverative recall errors. Executive functioning and selective attention were assessed with the Colour-Word Interference Score from the Stroop test (Golden 1978). Phonemic and semantic verbal fluency was assessed with the Controlled Oral Word Association Test (COWAT) (Benton A 1989), which can also be considered a measure of executive functioning. The neuropsychological test battery was administered by psychologists or advanced psychiatric nurses extensively trained and supervised in using the test battery. Experienced psychologists scored all the tests.

4.3 Ethical aspects

The genetic studies of the bipolar disorder were approved by the Ministry of Social Affairs and Health and the Ethics Committee of the National Public Health Institute. All patients and family members gave full informed consent and all blood samples were taken in accordance with the Helsinki Declaration and its amendments.

4.4 Laboratory methods and statistical analysis

Descriptions of the methods used in the present study can be found in the original articles (I–IV), as described in Table 11.

TABLE 11. Methods used in the present study

Material or Method	Original Publication
Study samples	
Family sample	I, II, III, IV
Late settlement sample	III
Control sample	I, III
Laboratory procedures and measurements	
DNA extraction	I, II, III, IV
DNA sample quality control	III
Spectrophotometry	III
PicoGreen measurements	I, III
Polymerase chain reaction (PCR)	I, II, III, IV
Whole genome amplification	I, II, III, IV
Agarose gel electrophoresis	I, II, III, IV
Sequenom MALDI-TOF Mass Spectrometry	I, II, III, IV
Multiply-primed rolling circle DNA amplification	III
Illumina genotyping system	III
Analysis programs	
Sequenom SpectroDesigner (version 1.3 and 2.0)	I, II, III, IV
Sequenom SpectroTyper RT (version 2.0)	I, II, III, IV
Pedcheck v.1.1	I, II, III, IV

Table continues

Table continues

Material or Method	Original Publication
Statistical methods and programs	
SAS 9.1 for windows	III
SNP selection	I, II, III, IV
MLINK/LINKAGE 4.1P	III
Analyze	II, III
Genehunter v2.1_r6	III
FBAT v.1.7.3. (HBAT)	I, II, III, IV
Haploview v4.0	II, III
Transmit 2.5.4.	I
QTDT	I, II
Simwalk 2.81	I, II, III
Pseudomarker	I

4.4.1 DNA extraction and quality control

DNA was extracted from Ethylenediaminetetraacetic acid (EDTA)-treated blood according to manufacturer's instructions with the Puregene DNA purification system (Gentra systems, Minneapolis, Minnesota, USA). Stock DNA samples were diluted in a concentration of 20 ng /µl using Tecan GenesisRSP150 (PLO, Espoo, Finland). The concentration of the diluted DNA was quantified with the Quant-IT Pico Green dsDNA Assay Kit (Eugene, Leiden, Netherlands) and corrected if necessary. We determined the sex of DNA samples with sex chromosomal markers and excluded all samples with discrepant results. Furthermore, we genotyped autosomal microsatellite markers to identify contaminated samples and confirm the relationships of the family members (unpublished data).

4.4.2 Candidate genes and marker selection

Study I focused on the *TSNAX/DISC1* gene cluster and study II on bipolar candidate genes; *BDNF, CREB1, NTRK2, SLC6A4, TPH2 and P2RX7*. Genotyped variants of the *TSNAX/DISC1* gene cluster are shown in Figure 6. In study II, all the variants of the genes investigated are listed in Table 12. In study III, the Illumina Panel IV (6K) Array, which includes 5663 SNPs distributed across the 22 autosomes and 345 SNPs in the X and Y chromosomes, was used. In study IV, the best associating SNPs

were selected from the Welcome Trust Case Control Consortium with p-values of $p < 0.000054$, from Baum et al. with p-value < 0.0001 and from their meta-analysis. Genotyped variants are listed in the Table 13.

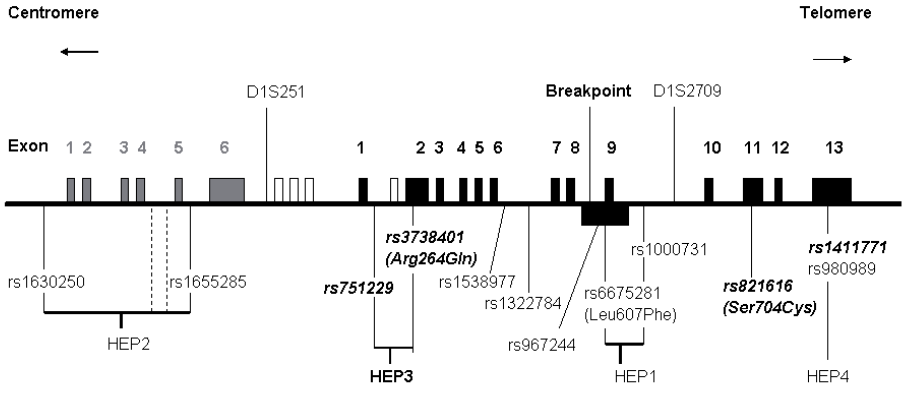

FIGURE 6. The schematic overview of the TSNAX/DISC1 region. The exonic structure of the TSNAX (grey boxes) and DISC genes (black boxes), and analyzed markers and haplotypes are shown in relationship to the intergenic exons (white boxes). Modified from Hennah et al. 2003. Reproduced with a permission of the copyright holder.

TABLE 12. Minor allele frequencies, alleles and positions of the analyzed candidate genes' SNPs (study II).

SNP	Tag SNP coverage[a]	Chr	Position	Location	Alleles[c]	MAF
BDNF	5/5 (1.0)					
rs11030096		11	27622119	3′ downstream	T/C	0.48
rs2203877		11	27627486	3′ downstream	T/C	0.48
rs6265		11	27636492	Exon (Val66Met)	C/T	0.14
rs11030102		11	27638172	Intron	C/G	0.24
rs11030108		11	27652040	Intron	G/A	0.34
rs6484320		11	27659764	Intron	A/T	0.17
rs2049046		11	27680351	Intron	T/A	0.47
rs7934165		11	27688559	Intron	G/A	0.48
rs1491850		11	27706301	5′ upstream	T/C	0.43
rs1491851		11	27709339	5′ upstream	C/T	0.42

Table continues

Table continues

SNP	Tag SNP coverage[a]	Chr	Position	Location	Alleles[c]	MAF
CREB1	2/3 (0.67)					
rs2709366		2	208200569	5′ upstream	C/A	0.19
rs2709376		2	208215894	5′ upstream	C/T	0.03
rs2709356		2	208237598	1. intron	C/T	0.14
rs2709359		2	208240643	1. intron	G/A	0.03
rs2709402		2	208246483	2. intron	C/A	0.13
rs10932201		2	208251763	4. intron	G/A	0.48
rs11904814		2	208252304	4. intron	T/G	0.35
rs2551921		2	208261061	6. intron	T/C	0.13
rs2709387		2	208267601	7. intron	G/A	0.14
NTRK2	3/31 (0.09)					
rs1147199		9	86465715	5′ upstream	G/A	0.17
rs1025743		9	86504937	1. intron	C/T	0.43
rs1187362		9	86528087	4. intron	A/T	0.25
rs1573219		9	86577442	10. intron	G/A	0.23
rs1047896		9	86615793	11. exon (3′ UTR)	A/G	0.19
rs11140767		9	86615874	11. exon (3′ UTR)	G/A	0.01
rs730240		9	86619183	11. exon (3′ UTR)	C/T	0.00
rs3654		9	86620311	11. exon (3′ UTR)	T/C	0.07
rs7038236		9	86678222	14. exon (3′ UTR)	C/A	0.14
rs11140793		9	86681300	14. exon (3′ UTR)	A/C	0.13
rs1221		9	86682430	14. exon (3′ UTR)	C/T	0.13
rs736744		9	86704227	14. intron	T/C	0.46
rs4410985		9	86799073	17. intron	T/C	0.42
rs729560		9	86824125	18. intron	A/G	0.42
rs3739570		9	86827398	20. exon (3′ UTR)	G/A	0.14
SLC6A4	4/4 (1.0)[b]					
rs7224199		17	25547852	3′ near gene	G/T	0.39
rs3794808		17	25555919	13. intron	C/T	0.37
rs140701		17	25562658	9. intron	C/T	0.37
rs140700		17	25567515	6. intron	C/T	0.11
rs6354		17	25574024	2. exon (5′ UTR)	T/G	0.19
rs4251417		17	25575984	1. intron	C/T	0.07
rs2020930		17	25590167	5′ upstream	C/T	0.02

Table continues

Table continues

SNP	Tag SNP coverage[a]	Chr	Position	Location	Alleles[c]	MAF
TPH2	8/10 (0.8)					
rs11178997		12	70618420	5′ near gene	T/A	0.08
rs10748185		12	70622122	2. intron	A/G	0.49
rs10879344		12	70630828	4. intron	A/T	0.02
rs1386494		12	70638810	4. intron	C/T	0.19
rs1386492		12	70648532	6. intron	T/C	0.25
rs1843812		12	70653922	7. intron	G/A	0.19
rs7305115		12	70659129	8. exon	G/A	0.46
rs7954332		12	70662605	8. intron	G/A	0.19
rs1386497		12	70678557	9. intron	A/C	0.20
rs12229394		12	70679181	9. intron	G/A	0.33
rs4760820		12	70683263	9. intron	C/G	0.36
rs10879354		12	70696049	9. intron	C/T	0.47
rs1487275		12	70696559	9. intron	A/C	0.39
rs4290270		12	70702502	10. exon	A/T	0.48
rs1872824		12	70716581	3′ downstream	G/A	0.37
P2RX7	6/13 (0.46)					
rs7310821		12	120051568	5′ upstream	C/T	0.09
rs591874		12	120055848	1. intron	A/C	0.34
rs1718125		12	120077402	2. intron	C/T	0.14
rs208290		12	120078439	4. intron	G/A	0.37
rs208294		12	120084636	6. exon (His155Tyr)	C/T	0.39
rs208298		12	120086621	6. intron	G/A	0.31
rs504677		12	120089572	8. intron	C/T	0.47
rs1718119		12	120099486	12. exon (Ala348Thr)	G/A	0.45
rs2230912		12	120106579	14. exon (Gln460Arg)	A/G	0.18
rs2303998		12	120139446	3′ downstream	G/A	0.00

[a] Tag SNPs defined by the multimarker option with cut off values of 0.2 for minor allele frequency and 0.8 for r^2 in the HapMap database.
[b] Cut off value of 0.1 for minor allele frequency.
[c] Major allele listed first.

TABLE 13. Minor allele frequencies, chromosomal positions and the replication sources of the analyzed SNPs (study IV).

Gene/Region	SNP	Chr	Replication	Alleles
1p31	rs2989476	1	WTCCC	G/C
TCF7L1	rs6732834	2	Baum et al.	C/T
DPP10	rs1375144	2	WTCCC	A/G
CMTM8	rs4276227	3	WTCCC	C/T
LAMP3	rs683395	3	WTCCC	A/G
SORCS2	rs4411993	4	Baum et al.	C/T
SORCS2	rs7683874	4	Baum et al.	G/A
SORCS2[a]	rs10937823	4	Baum et al.	C/T
6p21.1	rs6458307	6	WTCCC	C/T
GABBR2	rs3802477	9	Baum et al.	T/C
DFNB31[a]	rs10982256	9	WTCCC	C/T
DFNB31	rs942518	9	Baum et al.	A/G
JAM3	rs10791345	11	Baum meta	G/A
DGKH[a]	rs9315885	13	Baum et al.	T/C
DGKH	rs1170191	13	Baum et al.	G/A
NALCN	rs9513877	13	Baum et al.	G/A
TDRD9	rs11622475	14	WTCCC	C/T
A2BP1	rs10500336	16	Baum et al.	A/G
A2BP1	rs7204975	16	Baum et al.	C/T
PALB2	rs420259	16	WTCCC	A/G
16q12.2	rs1344484	16	WTCCC	T/C
PLCG2	rs4586425	16	Baum et al.	C/T
NXN	rs2360111	17	Baum et al.	C/T
FZD2	rs4792948	17	Baum et al.	C/T
SLC39A3[a]	rs4806874	19	Baum meta	A/G
CDC25B	rs3761218	20	WTCCC	T/C

[a] Major allele listed first.

4.4.3 Genotyping and quality control

This study utilized two different SNP genotyping methods. Primarily, SNP genotyping was performed using Sequenom's MassARRAY system (Sequenom Inc., San Diego, CA, USA) (I–IV). The primers for the SNP assays were designed using the SpectroDESIGNER software, versions 1.3 and 2.0 (Sequenom Inc., San Diego, CA, USA). SNP genotyping was performed following the manufacturer's guidelines using, either the homogenous Mass Extension (hME) reaction or the

iPLEX reaction on the Sequenom MassARRAY System (Sequenom Inc.). The second genotyping method was the commercial Illumina Linkage Panel IV (6K) Array (Illumina, San Diego, CA, USA) (II). Arrays were scanned with the illumina BeadStation 500GX scanner and analyzed with Illumina BeadScan Version 2. The genotyping sample included eight duplicates and eight water control samples. All genotypes were checked for correct Mendelian transmission using PedCheck v1.1. (O'Connell and Weeks 1998).

4.4.4 Statistical analysis

In study I, a single marker association test was performed using the pseudomarker program (Goring and Terwilliger 2000) and the 'association given no linkage' -option of the program. Haplotype analysis was conducted using the TRANSMIT 2.5.4. program (Clayton 1999) for all affected individuals, and separately for affected females and affected males.

In studies I and II, we analyzed the association of the variants with the neuropsychological test variables using the Quantitive Transmission Disequilibrium test (QTDT) program (Abecasis et al. 2000). Age, sex and the presence of the psychosis were used as covariates. 100 000 permutations were performed to obtain empirical p-values. To test the associating haplotypes and quantitative traits, the haplotypes were constructed for each genotyped individual using the SimWalk2 program (Sobel and Lange 1996).

In studies II and IV, the single marker association test was performed using the haplotype relative risk (HRR) test of the ANALYZE package (Lathrop and Lalouel 1984) and the family–based association test (FBAT) version 1.7.3. The haplotype extension (HBAT) of the FBAT program (Horvath et al. 2004) was used to test the association analysis of two – and three SNP haplotypes with the sliding window. The statistical comparisons of neuropsychologicals test results among selected genotyped groups were analyzed in SPSS 14.0 (SPSS Inc. Chigago, Illinois, USA) using one-way ANOVA.

In the genome-wide scan study (III), the data were analyzed using the AUTOGSCAN 1.0 program (Hiekkalinna and Peltonen 1999), which automates the use of the ANALYZE package (Lathrop and Lalouel 1984). An affected-only linkage analysis was performed with recessive and dominant models. Multipoint non-parametric linkage (NPL) analyses and haplotype analyses were conducted only for the regions showing the highest genome-wide significance using the Genehunter v2.1_r6 program (Kruglyak et al. 1996). Association analyses were conducted by FBAT version 1.7.3. using the single marker association test and the HBAT (Horvath et al. 2004) with the sliding window.

5 Results and discussion

5.1 Association analysis of the *DISC1* gene (I)

Bipolar disorder and schizophrenia have at least partially convergent aetiology, even though they represent separate disease entities with specific diagnostic criteria. They share several clinical features and cognitive impairments. In addition, both diseases are found to co-segregate in many families. Although psychosis is a primary diagnostic criterion for SCZ, a large part of BP-I cases have intermittent psychotic symptoms. There is, possibly, a more complex relationship between the psychotic disorders and thus they may share some genetic susceptibility loci. A majority of studies have found evidence of the role of the *DISC1* gene in psychotic disorders: SCZ, BP and schizoaffective disorder (Porteous et al. 2006). *DISC1* encodes an 854-amino-acid protein, which interacts with a variety of proteins known to be involved in several neuronal functions: cytoskeletal regulation, neuronal outgrowth and migration, and signal transduction (Kamiya et al. 2006; Morris et al. 2003). We attempted to identify whether *DISC1* has an aetiological role in the genetic background of psychotic and bipolar spectrum disorders and related quantitative cognitive traits in Finnish BP families. We also analyzed SNPs in the *TSNAX* gene next to *DISC1*.

We looked for association of allelic variants in the *TSNAX/DISC1* gene cluster using 13 SNPs across the 600-kb region in 723 individuals of 179 Finnish BP families. We used two diagnostic categories: psychotic disorder and bipolar spectrum disorder. Using psychotic disorder as an outcome, the strongest association was observed for the T-A haplotype of rs751229 and rs3738401 at the 5' end of *DISC1*. The haplotype was over-transmitted to males with psychotic disorder ($p = 0.008$) and a rare extension of the haplotype, including the T-A-G alleles of rs751229, rs3738401 and rs1538977, respectively, was over-transmitted in affected males ($p = 0.0007$ with both genders and $p = 0.007$ for males alone). This finding was consistent with earlier studies in Finnish schizophrenia families (Hennah et al. 2005; Hennah et al. 2003). Another allelic haplotype of the same variants was also overtransmitted to males ($p = 0.001$ for C-G-A allele combination).

With bipolar spectrum disorder as an outcome, we found a major haplotype of rs1630250 and rs1615409 within the *TSNAX* gene to be over-transmitted in affected females and males ($p = 0.007$ global and for G-C alleles $p = 0.007$). The signal for association was found to be derived from bipolar spectrum individuals without psychotic features ($p = 0.0014$ global and for females $p = 0.00001$). A haplotype at the 3' end of *DISC1* associated with bipolar spectrum disorder ($p = 0.0002$) with the T-T alleles of rs821616 and rs1411771 under-transmitted in both females and

males. An extended haplotype of rs821616, rs1411771 and rs980898 was under-transmitted in both genders with the T-T-G alleles (p = 0.0001). The signal for this haplotype was derived from individuals of bipolar spectrum disorders with or without psychotic features. These results support evidence of the association of common and rare haplotypes around the 5' end of *DISC1* with psychotic disorder in males. The results provide consistent evidence of association around the 5'end of *TSNAX* and the 3' end of *DISC1* with bipolar spectrum disorder.

We tested the association between the variants of the *DISC1/TSNAX* gene cluster and cognitive traits derived from the neuropsychological test battery in 158 individuals from the BP families. The traits were selected based on the previous findings for *DISC1*, high heritability estimates of these traits in Finnish BP families and their suggested validity as endophenotypes of BP. The risk haplotype for psychotic disorder including rs751229 at the 5' end of *DISC1* also associated to perseverations (p = 0.035; for rs751229 alone p = 0.0012) and long delay recall (p = 0.0032). A protective haplotype of rs1655285, rs751229 and rs3738401 at the 5' end of *DISC1* with G-T-G alleles was associated with auditory attention (p = 0.0059). The 3' end variants associated with several cognitive traits with the most robust signal between rs821616 and verbal fluency and rs980989 and psychomotor processing speed (p = 0.011 for both). Psychomotor processing speed is considered a strong and valid endophenotype for BP (Antila et al. 2007a; Antila et al. 2007b).

To conclude, these results support the involvement of *DISC1* in the genetic aetiology of BP and suggest that its distinct variants contribute to variation in the dimensional features of psychotic and bipolar spectrum disorders in Finnish BP families. The alternative associating haplotypes found in the same set of BP families gives evidence for allelic heterogeneity within *DISC1*, which eventually leads to heterogeneity in clinical outcomes as well. The variants included in these associating haplotypes also displayed association with different cognitive traits (Table 14), proposing possible systems underlying the biology of BP.

TABLE 14. The most significant association results of genes studied in this thesis.

Disorder	Gene	Variant or haplotype	No. of affected	Statistical method	p-value
Bipolar	BDNF[a]	rs1491851	227	FBAT	0.032
spectrum	SLC6A4	rs3794808	227	FBAT	0.045
disorder	SLC6A4	rs140700	227	FBAT	0.021
	P2RX7	rs2230912	227	FBAT	0.048
	BDNF	rs7934165-rs1491850-rs1491851	227	FBAT	0.039
	BDNF	rs7934165-rs1491850-rs1491851	227	FBAT	0.010
	BDNF	rs1491850-rs1491851	227	FBAT	0.004
	SLC6A4	rs7224199-rs3794808	227	FBAT	0.037
	SLC6A4	rs3794808-rs140701	227	FBAT	0.043

Table continues

Table continues

Disorder	Gene	Variant or haplotype	No. of affected	Statistical method	p-value
	SLC6A4	rs3794808-rs140701-rs140700	227	FBAT	0.029
	SLC6A4	rs140701-rs140700	227	FBAT	0.029
	SLC6A4	rs140701-rs140700-rs6354	227	FBAT	0.019
	SLC6A4	rs140700-rs6354	227	FBAT	0.017
	SLC6A4	rs140700-rs6354-rs4251417	227	FBAT	0.028
	P2RX7	rs208290-rs208294-rs208298	227	FBAT	0.032
	P2RX7	rs208290-rs208294-rs208298-rs504677	227	FBAT	0.006
	P2RX7	rs208290-rs208294-rs208298-rs504677	227	FBAT	0.024
	P2RX7	rs208294-rs208298	227	FBAT	0.039
	P2RX7	rs208294-rs208298-rs504677	227	FBAT	0.010
	DISC1[a]	rs1630250-rs1615409	227	TRANSMIT	0.007
	DISC1	rs1000731-rs821616	227	TRANSMIT	0.025
	DISC1	rs821616-rs1411771	227	TRANSMIT	0.004
	DISC1	rs6675281-rs1000731-rs821616	227	TRANSMIT	0.033
	DISC1[a]	rs821616-rs1411771-rs980989	227	TRANSMIT	0.021
Psychotic	BDNF	rs2049046-rs7934165-rs1491850	251	FBAT	0.015
disorder	BDNF	rs7934165-rs1491850	251	FBAT	0.015
	BDNF	rs7934165-rs1491850-rs1491851	251	FBAT	0.024
	NTRK2	rs1147199-rs1025743-rs1187362	251	FBAT	0.039
	SLC6A4	rs3794808-rs140701-rs140700	251	FBAT	0.033
	SLC6A4	rs140701-rs140700-rs6354	251	FBAT	0.048
	P2RX7	rs208290-rs208294-rs208298	251	FBAT	0.027
	P2RX7	rs208290-rs208294-rs208298-rs504677	251	FBAT	0.011
	P2RX7	rs208294-rs208298	251	FBAT	0.049
	P2RX7	rs208294-rs208298-rs504677	251	FBAT	0.032
	P2RX7	rs208294-rs208298-rs504677	251	FBAT	0.021
	P2RX7	rs208294-rs208298-rs504677	251	FBAT	0.040
	P2RX7	rs1718119-rs2230912	251	FBAT	0.045
	P2RX7	rs1718119-rs2230912-rs2303998	251	FBAT	0.047
	DISC1	rs1655285-rs751229	251	TRANSMIT	0.459
Broad	SORCS2	rs4411993	298	FBAT	0.014
mood	SORCS2	rs7683874	298	FBAT	0.008
disorder	SORCS2	rs10937823	298	FBAT	0.004
	DFNB31	rs10982256	298	FBAT	0.001

[a] Gene and variant associates also with cognitive traits.

5.2 Association analysis of six candidate genes (II)

As discussed in the previous sections, linkage and association studies over the past years have highlighted several genomic regions of interest and focused attention on several potential candidate genes for bipolar disorder. However, none have yet been shown to be causative as have been for many non-psychiatric disorders (Craddock and Forty 2006; Serretti and Mandelli 2008). Recent GWAS and other studies in BP have strongly suggested that BP can result from the effects of multiple rare variants and that there are few, if any, common variants with large effect sizes on bipolar disorder risk (Ferreira et al. 2008; Scott et al. 2009). Therefore, genetic studies in isolated homogenous populations, such as the Finnish population, may be advantageous in identifying rare variants for BP. Other approaches are also required to disentangle conflicting findings, such as gene interaction analyses and endophenotype investigations. It is unclear which phenotype best captures the underlying mechanism of BP, and as a result the use of different phenotypic subtypes or endophenotypes, such as cognitive traits or the presence of psychotic symptoms, may be more appropriate phenotypes for study than DSM-diagnostic categories.

We wanted to examine in a family-based sample the roles in the genetic background of bipolar and psychotic disorders of the widely studied candidate genes from three seemingly distinct signalling systems that play regulatory roles in many neuronal functions: serotonin-related genes (*SLC6A4* and *TPH2*), BDNF-related genes (*BDNF*, *CREB1* and *NTRK2*) and one gene related to the inflammation and cytokine system (*P2RX7*). In addition to diagnostic phenotypes, variants were tested to determine whether these candidate genes played possible aetiological roles in the genetic background of any related quantitative cognitive traits in Finnish BP families (Antila et al. 2007a).

We investigated association of a total of 68 SNPs in the 723 individuals of the 180 Finnish BP families. Consistent with earlier findings in other populations and samples, the functional variant Val66Met (rs6265) of *BDNF* was found to show a trend of association with bipolar spectrum disorder ($p = 0.062$; for only familial cases $p = 0.016$) as well as the variant at the 5'end of *BDNF* ($p = 0.032$ for rs1491851). The haplotype at the 5' end of *BDNF* associated with bipolar spectrum disorder ($p = 0.004$ for haplotype T-C of rs1491850 and rs1491851, respectively). Association was seen between two variants of *SLC6A4* ($p = 0.045$ for rs3794808 and $p = 0.021$ for rs140700) and BP spectrum. However, the association was stronger in males than for the both genders ($p = 0.007$ for rs140700). Haplotype T-G of rs140700 and rs6354 within *SLC6A4* was associated stronger in males with bipolar spectrum disorder ($p = 0.004$; for both genders $p = 0.017$). In addition, a variant rs2230912 within *P2RX7* associated with BP spectrum ($p = 0.048$ with both genders, $p = 0.038$

for males). A four-SNP protective haplotype G-T-G-C of rs208290-rs208294-rs208298-rs504677 showed the strongest association (p = 0.006 with both genders).

We tested the association between the same variants that associated with dichotomous traits and quantitative traits derived from the neuropsychological test battery in 158 individuals from the bipolar families. The allele Val66 of *BDNF* associated with better performance in retention (p = 0.003) as well as the T-C haplotype of rs1491850 and rs1491851 (p = 0.0004). For the *P2RX7* gene, a four-SNP protective haplotype G-T-G-C of rs208290-rs208294-rs208298-rs504677 also showed relatively strong association with better performance in executive functions, and semantic and phonemic fluency (p = 0.006 and p = 0.0003, respectively). Better performance in semantic fluency was also associated with the C allele of rs504677 (p = 0.0001). The variants in the 14th exon of *NTRK2* associated with the most robust signal for recognition memory (rs7038236, rs11140796 and rs1221, p = 0.0065, p = 0.005 and p = 0.0049, respectively) and auditory attention (rs1025743, p = 0.011).

The expression level of *BDNF* increases after antidepressant treatment (Duman and Monteggia 2006; Nibuya et al. 1995; Russo-Neustadt et al. 1999), which is the primary reason to suggest that it is a potential susceptibility gene for mood disorders. Association studies have repeatedly supported the evidence that *BDNF* is a susceptibility gene for mood disorders (Serretti and Mandelli 2008). Most studies have shown overtransmission of the common Valine allele (Geller et al. 2004; Green et al. 2006; Lohoff et al. 2005b; Neves-Pereira et al. 2002; Sklar et al. 2002; Strauss et al. 2004), but some studies have not detected association (Schumacher et al. 2005). Previous studies have strongly implicated a role of *BDNF* in cognitive functions, particularly in memory acquisition and consolidations (Martinowich and Lu 2008; Pang and Lu 2004; Tyler et al. 2002; Woo and Lu 2006). In the present study, we managed to replicate the association of the Valine allele with both BP and better performance in a memory test. The Val66Met variant lies within a large haplotype block, and thus it is difficult to identify which variant (possibly many) within the block is causative. The contradicting findings with the variants within *BDNF* suggest that this gene may contribute to specific aspects of BP, such as cognitive functions. Interestingly, we found that the *NTRK2* gene, which is known as a BDNF-related gene, shows minor evidence of association with BP and recognition memory, further supporting the involvement of the BDNF signalling system in the genetic background of both bipolar and cognitive functions. However, *CREB1* did not reveal any evidence of association with BP or cognitive functions.

Serotonin-related genes, *SLC6A4* and *TPH2*, are consistently associated with BP (Cho et al. 2005; Cichon et al. 2008; Grigoroiu-Serbanescu et al. 2008; Harvey et al. 2007; Harvey et al. 2004; Lopez et al. 2007; Roche and McKeon 2009; Van Den Bogaert et al. 2006). However, we did not found evidence of association with BP for the two functional variants of *SLC6A4* and *TPH2*. Nonetheless, evidence of the association of a single variant and the two-SNP haplotype within *SLC6A4*

was found among BP males. In the present study, serotonin-related genes indicated association with the verbal working and immediate memory cognitive functions.

We found significant associations of the variants within *P2RX7* with BP and executive functions, particularly with semantic and phonemic fluency, which is proposed to be a good endophenotype for BP. Executive functions are found to be impaired in euthymic depression patients, and thus our finding may imply that variants within the gene associate with the depressive symptoms of BP patients. The function of *P2RX7* in the central nervous system is only partly known. It has a known role in neurotransmitter release and inflammatory responses. It has been suggested that symptoms of depression are associated with increased immune activity and consequently the role of *P2RX7* in BP might be more related to its immune function (Humphreys and Dubyak 1998).

This study supports the involvement in Finnish BP families of *BDNF, SLC6A4* and *P2RX7* in the genetic aetiology of BP by replicating previous findings as well as in the genetic background of cognitive traits. *BDNF* and its receptor *NTRK2* associate with verbal learning and memory and *P2RX7* with executive function or fluency of thinking.

5.3 Genome-wide linkage analysis

We conducted a genome-wide linkage scan for broad mood disorder using 5554 autosomal SNPs in a family sample of 23 pedigrees originating from the late settlement region in Finland. Our aim was to identify loci that harbor susceptibility genes for broad mood disorder in our relatively homogenous set of BP families. We knew that we would not identify common risk variants, but hoped to find high risk variants that might be population specific. There were two regions, 9p13 and 7q31, that exceeded the LOD score of 3.0 (Z = 4.02 with rs716933 on 9p13, and Z =3.20 with rs1510504 on 7q31). These chromosomal regions that exceeded a LOD score of 3.0 were analyzed by a haplotype and association analysis. For further linkage and association analyses, we used a larger set of 179 pedigrees including the initial 23 families originating from the late settlement region.

In the initial sample of 23 families, the second best linkage result was rs1510504 at 7q31.31 with a LOD score of 3.20. Furthermore, suggestive linkage was observed with the flanking markers rs1880180 (a LOD score of 2.06) and rs1419438 (a LOD score of 2.17), respectively 4.5 Mb and 8.2 Mb downstream from marker rs1510504. Eleven families originating from the late settlement region had a major influence on the linkage signal. The maximum multipoint NPL score using the set of six markers on 7q22.3-7q31.32 yielded a LOD score of 2.83. In the association analysis, marker rs342296 provided evidence of association (p = 0.00014). In addition, across the 7q22.2-7q35 region a two-SNP haplotype association revealed a risk haplotype A-G for rs1034761 and rs342296 (p = 0.0004, Table 15).

Potential bipolar candidate genes on the linkage region of chromosome 7 include calcium-dependent activator protein for secretion 2 (*CADPS2*) and potassium-voltage-gated channel, member 2 (*KCND2)*. CADPS2 is expressed in the nervous system, particularly in the cerebellum (Cisternas et al. 2003). Interestingly, cadps2 knockout mice have been observed to have defects in sleep-wake transitions and circadian rhythms. Sleep-wake cycles are often disrupted in BP and may be caused by the abnormalities in the circadian clock (Shi et al. 2008a). In addition, Cadps2 is involved in the release of neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) from neocortical and cerebellar neurons (Sadakata et al. 2007a; Sadakata et al. 2007b). *KCND2* encodes a protein that forms voltage-activated A-type potassium ion channels and is prominent in the repolarization of the action potential. It also has a role in the regulation of neurotransmitter release and in neuronal flexibility (Isbrandt et al. 2000; Zhu et al. 1999)

In the initial sample of 23 families, the most significant two-point LOD score (LOD = 4.02) was obtained at 9p13 with marker rs716933. Families from the late settlement region primarily contributed to the linkage, including the extended pedigree with the largest signal (LOD = 1.27). A maximum NPL score of 3.02 was revealed at marker rs716933 with a 7.8 cM interval around the marker showing a LOD score > 2.7. The linkage region spans the centromeric region, which contains intra- and interchromosomal duplications, including large blocks of duplications and a heterochromatin block (Humphray et al. 2004). Therefore, it is impossible to identify which side of the centromere the susceptibility region lies using the current set of markers. The centromeric region of the chromosome 9 harbors potential candidate genes, such as contacting associating protein-like 3 (*CNTNAP3*) and aldehyde dehydrogenase 1B1 precursor (*ALDH1B1*). *CNTNAP3* encodes contactin-associated protein 3 (Caspr3), which is a member of the neurexin superfamily that mediates neuron-glia interactions (Spiegel et al. 2002; Traut et al. 2006) and is expressed in the nervous system; cerebellum, caudate nucleus, hippocampus and sustantia nigra (Spiegel et al. 2002). The *ALDH1B1* gene encodes aldehyde dehydrogenase protein, which belongs to the aldehyde dehydrogenase protein family that plays a major role in the detoxification of alcohol derived acetylaldehyde and is involved in the metabolism of corticosteroids and neurotransmitters (Vasiliou and Nebert 2005). Furthermore, the protein family is upregulated as part of the oxidative stress response, which has been implicated in the pathophysiology of BP (Machado-Vieira et al. 2007). In the haplotype association analysis a risk haplotype A-G of rs1220087 and rs666478, 1.11 Mb downstream from rs716933, showed the strongest signal (p = 0.00001). The region includes five genes, of which Phospholipase A2-activating protein (*PLAP*) regulates inflammatory response through its activation of phospholipase A2 (*PLA2A*), which was previously associated with BP in Caucasian families (Dawson et al. 1995; Jacobsen et al. 2001).

Six markers from the chromosome 7 linkage region and seven markers from the chromosome 9 linkage region were genotyped in a complete countrywide BP family sample, which comprised of 576 new individuals for a total of 723

individuals. For chromosome 7, the linkage signal declined to LOD <1.5 and the multipoint NPL score declined to NPL 1.40. The signal was thus derived mainly from the late settlement region families. For chromosome 9, a LOD score of 3.06 was obtained with marker rs716933 using the complete set of families. However, no additional families showed strong evidence for linkage.

In conclusion, we identified two chromosomal regions on 7q22.3–7q31.32 and 9p21.3–9q13, of which 7q31 is a novel finding for broad mood disorders. Further analyses of the best markers in the complete set of 179 BP families supported the findings on chromosome 9p13. Because the majority of the linkage signal came from the late settlement families, including two extended families, and the signal was not replicated in the larger family sample, the signal may result from a rare variant with a large effect size in the locus. The 9p13 region harbors several interesting candidate genes for mood disorders, including *CNTNAP3* and *ALDH1B1*.

TABLE 15. Haplotype association results in 23 late settlement families for the 7q22.2–7q31.33 and 9p22.2–q21.13 chromosomal regions. Results are given for the haplotype that gave the most significant P-value for each SNP pair. Bold SNP markers indicate a LOD >1.0 and bold p-values indicate p < 0.01.

Chromosome 7q22.2–7q31.33					
SNPs[a]	**Distance (Mb)[b]**	**Haplotype**	**Frequency**	**Z-value[c]**	**P-value[d]**
rs234–rs41261	105.15–105.20	AA	0.233	2.688	**0.007**
rs41261–rs176481	105.20–105.32	AG	0.323	2.768	**0.005**
rs1024761–**rs342296**	10.568–105.96	AG	0.206	3.538	**0.0004**
rs342285–rs1476878	10596–106.29	GG	0.224	2.918	**0.003**
rs1476878–rs257376	106.29–106.39	GA	0.194	2.409	0.016
rs43077–rs2040587	111.83–114.26	GG	0.214	2.222	0.026
rs2040587–rs2056865	114.26–115.81	AG	0.141	-2.183	0.029
rs868053–rs12217	120.37–121.30	AG	0.261	2.013	0.044
Chromosome 9q22.2–9q21.13					
SNPs[a]	**Position (Mb)[b]**	**Haplotype**	**Frequency**	**Z-value[c]**	**P-value[d]**
rs1327063–rs702223	22.35–23.82	CC	0.241	-2.184	0.028
rs757099–rs1220087	25.72–25.77	AG	0.462	-1.978	0.047
rs1220087–**rs666478**	25.77–27.16	AG	0.225	4.405	**0.00001**
rs666478–rs1555454	27.16–27.21	GA	0.506	3.218	**0.001**
rs1475656–rs1412340	30.9–31.6	AA	0.139	-2.063	0.0.39
rs1412340–**rs288886**	31.6–31.73	GA	0.149	2.404	0.016
rs288886–rs671018	31.73–32.55	GG	0.329	-2.202	0.027
rs671018–rs855543	32.55–33.63	GA	0.246	-2.776	**0.005**
rs855543–rs471371	33.63–33.86	GA	0.279	2.099	0.035
rs1138374–**rs716933**	37.96–38.32	GA	0.215	3.230	**0.001**
rs716933–rs1004604	38.32–38.35	GA	0.340	-2.655	**0.007**
rs1004604–rs987187	38.35–38.36	AG	0.569	-2.101	0.035
rs1074670–rs1404195	71.24–71.61	GA	0.200	2.147	0.031

[a] Haplotypes were calculated by the sliding window method of 2-SNPs. Associating haplotypes of 2-SNPs were analyzed as extended 3-SNP haplotypes. Haplotypes were analyzed between markers giving LOD scores above1.5 (in bold) as well as five flanking markers downstream and upstream of the significant marker.
[b] The position of the SNP is according to the May 2004 human reference sequence (NCBI Build 35)
[c] A negative Z-statistic score indicates a protective haplotype and positive score indicates a risk haplotype.
[d] FBAT p-values are obtained with the HBAT option.

5.4 Genome-wide association replicate analysis (IV)

Recent genome-wide association studies have provided multiple variants that yielded consistent association with complex disorders, such as type 2 diabetes (The Wellcome Trust Case Control Consortium 2007). Three groups have performed independent GWAS of bipolar disorder using either pooled (Baum et al 2008) or individually genotyped samples (The Wellcome Trust Case Control Consortium 2007, Sklar et al.2008). However, there has been a lack of overlap between these studies for their most significant results; only a small amount of findings are shared (Baum and Hamsphere 2008). Part of the lack of overlap can be explained by different study designs, genetic heterogeneity difficulties in detecting rare alleles and/or the small effects of variants on BP risk. In addition, difficulties in diagnosis and the lack of specific quantitative biological markers render mapping more challenging. In this study our aim was to evaluate the role of associating variants from three bipolar GWA studies in Finnish bipolar families.

We have analyzed in 723 individuals of 180 Finnish BP families the most significantly associating variants from the Wellcome Trust Case Control Consortium (The Wellcome Trust Case Control Consortium 2007), Baum et al. (Baum et al 2008) and a meta-analysis (Baum and Hamsphere 2008). The most strongly associating SNPs of p-values $p < 0.0001$ and $p < 0.000054$ were selected from Baum et al. and WTCCC, respectively. SNPs located in the lithium pathway genes were also selected for the present study. Altogether 26 SNPs were genotyped and analyzed using two diagnostic categories: bipolar type I disorder and broad mood disorder i.e. bipolar type I, II, NOS and cyclothymia.

We found consistent evidence supporting the previously reported *DFNB31, SORCS2, SCL39A4* and *DGKH* findings. For both diagnostic categories, we found three SNPs in *SORCS2* associating significantly ($p = 0.014–0.0041$). Two of these variants were allelic replications and represented the same signal according to haplotype analysis. *SORCS2* is expressed in the central nervous system and encodes a receptor in the synaptic signal system. The best association was found with one variant of *DFNB31* ($p = 0.00012$ for broad mood disorder and $p = 0.0097$ for bipolar type I disorder) that remained significant after correction for multiple testing. *DFNB31* is also strongly expressed in the central nervous system and is involved in synaptic transmission. Interestingly, mutation in this gene causes a recessive form of hearing loss. With bipolar type I disorder as an outcome, we found a signal for association with an allelic variant of *SLC39A4* ($p = 0.034$). However, we did not find support for the *PLAB2* association, which was the most significant result in the WTCCC GWAS.

In conclusion, this replication revealed significant association with four genes previously associated with BP, and thus supports their role as predisposing genes

in the aetiology of BP and other psychiatric diseases. The present replication may give us a hint of the pathways involved in the development of bipolar and related psychiatric disorders.

6 Concluding remarks and future prospects

BP has a strong genetic component. Nonetheless, the underlying genetic and pathophysiological inducers of the disorder remain elusive. Intensive genetic research of BP, including multiple linkage and candidate gene studies, have revealed several regions of interest as well as evidence implicating particular genes, but no loci or genes have been convincingly identified. The pessimistic view, that psychiatric genetics is so complex that advances are unlikely, has been common in the past decades. However, in the last few years numerous improvements in the molecular genetics research, including technical and statistical developments, have facilitated the studies of complex disorders. The aim of this thesis was to investigate the genetic basis of BP and to identify genetic variants that increase susceptibility to BP and its related cognitive endophenotypes in the Finnish families. In reference to the aims of this thesis study, the following findings are presented:

1. In the association study of the *TSNAX/DISC1* gene cluster, support was found for the involvement of *DISC1* in the genetic aetiology of BP. Distinct allelic variants contribute to variation in the psychotic and bipolar spectrum disorders in Finnish BP families. The variants included in these associating haplotypes also displayed association with different cognitive traits, proposing possible systems underlying the biology of BP.

2. The association study supports the involvement of *BDNF, SLC6A4* and *P2RX7* in the genetic aetiology of BP through replication of previous findings. The genes also showed contribution to the genetic background of cognitive traits in Finnish BP families. *BDNF* and its receptor *NTRK2* associate with verbal learning and memory and *P2RX7* with executive function or fluency of thinking.

3. In the genome-wide linkage scan, two chromosomal regions on 7q22.3-7q31.32 and 9p21.3-9q13 showed evidence for linkage. Chromosome 7q31 appears to be a novel finding for broad mood disorders. Further analyses in the complete set of BP families supported the findings on chromosome 9p13. The majority of the linkage signal came from the late settlement families, suggesting that the signal may result from a rare variant with a large effect size in these families.

4. In the genome-wide association replication analysis, six associating SNPs in the *DFNB31, SORCS2, SLC39A3* and *DGKH* genes replicated the recent GWAS findings in the Finnish BP families. These findings further support the involvement of these genes and their pathways in the biology of BP.

The most promising linkage findings on chromosome 9p13 in the present study suggest the identification of a BP susceptibility region. A future task is to define the underlying causative variants in that highly duplicated region by more sophisticated CNV analysis and sequencing the most promising regions. In addition, the promising association findings of the *DISC1/TSNAX*, *DFNB31 and SORCS2* genes in the present study suggest involvement of these genes in the aetiology of BP. Therefore, sequencing these genes may expose causative variants in the Finnish population. Recent genome-wide studies have identified many common variants that are associated with complex disorders, providing new opportunities to explore their biological bases. For BP, GWAS and other studies strongly suggest that BP results from the effects of multiple rare variants and that there are only a few common variants that can be unequivocally identified in various study populations. Allelic heterogeneity has the potential to have important roles in the genetic aetiology of disorders, but it is difficult to detect in population based-samples using the common variants often genotyped. In order to fully understand the allelic heterogeneity that underlies common diseases, complete genome sequencing for many individuals with and without disease is required. Therefore, it is likely that most of the genetic variants increasing risk for BP remain to be discovered.

The identification of risk variants has the potential to help improve the understanding of BP pathophysiology. The roles of epigenetic factors, rare variants and copy number variants are important targets for future BP genetic research. Since epigenetic mechanisms increase the complexity of genomic responses by allowing for both short- and long term modifications of the genome, they provide a mechanism for preserving information on environmental exposures. BP may be due to a combination of multiple common variants with relatively small effect sizes, a few rare variants with larger effect sizes, structural variations such as CNVs and epigenetic modifications. The identification of susceptibility genes will also facilitate the development of treatments better targeted at the biochemical level. In addition, it will further facilitate the identification of environmental factors that alter risk. Once the aetiological factors are characterized, it may be possible to provide genetically- and environmentally-tailored occupational, social and psychological advice to individuals with a high risk of mood disorders.

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